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March 7, 1966

This report of the 19th Tobacco Chemists' Research Conference is being issued although it is complete only through Paper #13. The remainder of the report will be issued when it is ready.

*D. P. Murrill*  
D. P. Murrill

Report of the  
19TH TOBACCO CHEMISTS' RESEARCH CONFERENCE

October 26-28, 1965

Lexington, Kentucky

D. P. Murrill, Editor

Richmond, Virginia

January 10, 1966

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PHILIP MORRIS INCORPORATED

Research Center

Report of the  
19TH TOBACCO CHEMISTS' RESEARCH CONFERENCE

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## INTRODUCTION

This report on the Nineteenth Tobacco Chemists' Research Conference, held in Lexington, Kentucky, October 26-28, 1965, contains abstracts of the papers presented and reviews by members of the Philip Morris Research Center who attended the Conference. The abstracts are taken from the official program of the Conference. The tables and diagrams used in the reviews are taken from slides which were presented with the papers.

If a reprint of a paper is available, no review is included in this report. Instead, the statement that a copy of the paper is available in the library is made at the end of the abstract.

Thirty-five papers were presented, twenty-nine from the United States, four from the United Kingdom, one from Switzerland, and one from Germany.

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## SUMMARY

F. E. Resnik

The University of Kentucky was the host for the 19th Tobacco Chemists' Research Conference at Lexington, Kentucky, on October 26-28, 1965, under the chairmanship of Dr. Charles Bortner. Thirty-five papers were presented to 230 scientists from nine countries.

The meeting was opened with a welcoming address by Dr. John W. Oswald, president of the University of Kentucky. He summarized the progress made at the University which, in its Centennial year, has an enrollment of over 18,000 students.

The symposium on the Biochemical Aspects of the Growth and Development of Tobacco Suckers was organized by T. C. Tso of the United States Department of Agriculture. The nine papers presented during the symposium covered three areas: patterns of growth, kinetics of growth and enzyme action, and patterns of inhibition. It was readily apparent that much has been learned about sucker growth and inhibition that should lead to new chemicals for control.

In the area of tobacco biochemistry, J. A. Weybrew of North Carolina University showed large differences in the amino acids when tobaccos were harvested at different stages of ripeness and subsequently cured at different rates. The amino acid contents also varied with regard to stalk position. C. F. Hinz of General Cigar discussed the microbial degradation of nicotine, and H. Silberman of Philip Morris presented data on the enzymatic degradation of the structural carbohydrates in selected tobaccos.

Studies of tobacco composition revealed the presence of two carboxylic acid precursors of solanone (2-methyl-5-isopropyl-1, 3-nonadien-8-one) from burley tobacco. The precursors were diastereoisomers of 4,8-dimethyl-6,8-dihydroxy-11-isopropyl-14-keto-4,9-pentadecadienoic acid.

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New technology resulted in an instrument for the measurement of moisture in cut tobacco by means of microwave absorption. J. O. Pullman of Liggett & Myers described the use of this equipment on the manufacturing floor and speculated on its use for moisture measurement on a single cigarette. A rapid method for the measurement of glycyrrhizin in licorice flavoring by infrared spectrophotometry was described by R. J. Morris, Jr. from MacAndrews & Forbes Company.

R. E. Thornton of the British-American Tobacco Company discussed pyrolysis techniques, results from which led to predictions regarding the precursors of phenols in cigarette smoke. Chlorogenic acid was suggested as a major contributor to smoke phenols as determined by pyrolysis techniques. A. W. Spears of P. Lorillard showed that C<sup>14</sup>-labeled glucose added to cigarettes also gave rise to phenol in cigarette smoke.

Cigarette smoke filtration again commanded considerable interest at the conference. The effect of the pH of cigarette smoke and of the filter on selective filtration of nicotine and of volatile acids was discussed by H. G. Horseywell of British-American Tobacco Company. M. L. Reynolds of Imperial showed the contribution and relative importance of filtration, re-pyrolysis, ventilation, and diffusion to the delivery of various smoke components. The measurement of the surface area of fibrous filters was described by C. H. Keith of Celanese Corporation. An interesting theoretical paper on the aspects of the fluid dynamics of cigarette smoke filtration was presented by W. George of Celanese Corporation. The critical Reynolds number for flows around obstacles in open, porous beds such as a cigarette filter was discussed. M. F. Kranc of Pittsburgh Activated Carbon Company gave a paper on the determination of the selective efficiency of charcoal in which he considered such variables as pore size distribution, activity level, apparent density, raw material, and impregnants.

Papers on the analysis of cigarette smoke dominated the

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conference. The papers ranged in subject matter from large scale, fully automated smoking machines, presented by R. E. Lang of American Machine & Foundry and F. Seehofer of Cigaretten-Fabriken, to the analysis of benzo(a)pyrene in the smoke from one cigarette, presented by B. W. Oliver of Tennessee Eastman Corporation. This benzo(a)pyrene method resulted in poor recoveries because of the numerous thin-layer chromatographic scans required for purification prior to spectrofluorimetric analysis. Recoveries were corrected by using C<sup>14</sup>-labeled benzo(a)pyrene. K. Grob of the University of Zurich showed the advantages of the use of glass capillary columns. He emphasized the necessity for analyzing fresh smoke, that is, within 0.1 to 0.2 second after formation. A gas chromatographic method for the determination of water in cigarette smoke, using pyridine as a solvent, was presented by F. A. Thome of Reynolds Tobacco Company. The gas chromatographic determination of menthol, propylene glycol, nicotine, and triacetin on a single chromatogram, using temperature programming, was presented by L. A. Lyerly of Reynolds Tobacco Company. The use of 3-methylbenzothiazolone hydrazone hydrochloride for the determination of aliphatic aldehydes in cigarette smoke was discussed, as was the use of ion exchange resins for the determination of cation and anion concentrations in tobacco and smoke. Benzyl benzoate and some ketones and phytol esters were identified in cigarette smoke. The ciliastatic components of the gas phase of cigarette smoke was studied by T. R. Walker of Tennessee Eastman Corporation. Cigarette smoke was separated by gas chromatography, and the eluted components were passed across a clam gill for cilia response. The gas chromatographic curves were then superimposed on the ciliastasis curves to ascertain the active components in cigarette smoke.

Dr. R. Bard, Assistant Vice President for Research and Development at the University of Kentucky, discussed at the

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banquet the "Tobacco-Health Program at the University of Kentucky." The program is being coordinated with the United States Department of Agriculture and involves the Agriculture, Chemistry, and Medical Departments at the University. The program will be concerned with the growing of tobaccos of different chemical compositions, analysis of the smoke from these tobaccos, followed by extensive animal testing.

The meeting in 1966 will be held in Winston-Salem, North Carolina, with Wake Forest College as host. The following year the meeting will be sponsored by Duke University of Durham, North Carolina.

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GROWTH AND DEVELOPMENT OF TOBACCO  
SUCKERS FOLLOWING TOPPING  
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Service, Crops Research Division,  
North Carolina State of the Uni-  
versity of North Carolina, Raleigh,  
North Carolina

### ABSTRACT

When Nicotiana tabacum cv. flue-cured is in flower the axillary buds are in various stages of development. In the axils of those leaves below the point of topping, there may be as many as three buds; primary, secondary, and/or tertiary. In leaf axils lower on the stalk, the number and stage of development of the buds are usually reduced. Topping, which destroys the apical dominance exerted by the growing point, stimulates growth of the axillary buds. The uppermost primary buds develop noticeably and establish a semblance of dominance. Removal of these buds (suckers) again will destroy apical dominance, and again the uppermost primary buds below these removed will develop noticeably. The appearance of secondary buds may occur following the removal of the primary buds in any given leaf axil. The pattern of development of suckers is probably due to the stage of development found in the various leaf axils at the time of topping.

### REVIEW BY G. H. BURNETT

In the general practice of tobacco growing, the flower of the plant is removed (topped). This causes the buds in the axils of the leaves just below the flower to increase in size. The removal of these buds (suckering) increases top leaf size and increases the nicotine content. The author pointed out that each time the plant is topped or suckered the physical balance of the rest of the plant is changed.

Through several diagrams and slides of plants, Dr. Decker showed the regrowth of these axillary buds over several weeks and after a varying number of suckerings.

In one experiment, eleven days after topping, the buds on the top three nodes had grown approximately 20 cm, whereas on the 4th to 15th nodes they had grown only 2 cm.

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Another experiment showed that the next top three nodes, i.e., nodes 4 to 6, grew from approximately 2 cm at the time of suckering to 12 cm seven days after suckering. After seven more days they had grown to 25 cm. There was an increase in the length of nodes 7 to 14, but it was of small significance.

The author also showed how the next three nodes, i.e., nodes 7 to 9, again increased in size after the plant had been suckered a second time. Size increases were from 4 cm to 9 cm to 14 cm to 28 cm, respectively, seven, fourteen, and twenty-one days after the second suckering.

THE FREE AMINO ACIDS OF SUCKER  
BUDS AND YOUNG LEAVES

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State of the University of  
North Carolina, Raleigh, North  
Carolina

ABSTRACT

Apical sucker buds and progressively larger (and older) leaves have been analyzed for "free" amino acids (i.e., soluble in 1/100 HCl) to establish distribution patterns in actively growing tissues. In general, concentrations were highest in the 1" leaflets and subsequently became "diluted" by growth. Relative to normal tissues, MH 30-suppressed suckers contained very much higher levels of amino acids in aggregate, but the distribution was disproportionate. Certain constituents, especially proline, asparagine, glutamine, aspartic acid, ammonia, histidine,  $\alpha$ -aminoadipic acid, and galactosamine, were higher by a factor of at least 3, while the levels of others such as glycine, alanine, valine, leucine, isoleucine, and  $\gamma$ -aminobutyric acid were either about normal or lower. This differential accumulation suggests abnormal metabolism rather than merely arrested growth. By contrast, N-deficient seedlings were very much lower in free amino acids, but the relative amounts were proportional.

REVIEW BY H. D. MERWIN

Apical sucker buds and progressively older leaves have been analyzed for "free" amino acids to establish distribution patterns in actively growing tissues.

A Spinco autoanalyzer was used to analyze the extract (1/100 HCl) from dried, ground material. The exact sequence of amino acids on the chromatogram is dependent on the instrument used. The method employed ninhydrin for development of the color, so positive ninhydrin substances other than  $\alpha$ -amino acids showed up.

The "free" amino acids were those in a pool of amino acids which were by-products of the Krebs cycle. This pool could furnish amino acids for protein formation and contained, also, acids left over from protein synthesis.

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Apical buds were analyzed, as were leaflets of various sizes. The older the leaves, the lower was the amino acid content with similar distribution of various acids. A similar effect was found in nitrogen-starved plants where the amino acid content was reduced and distributions were similar to normal plants.

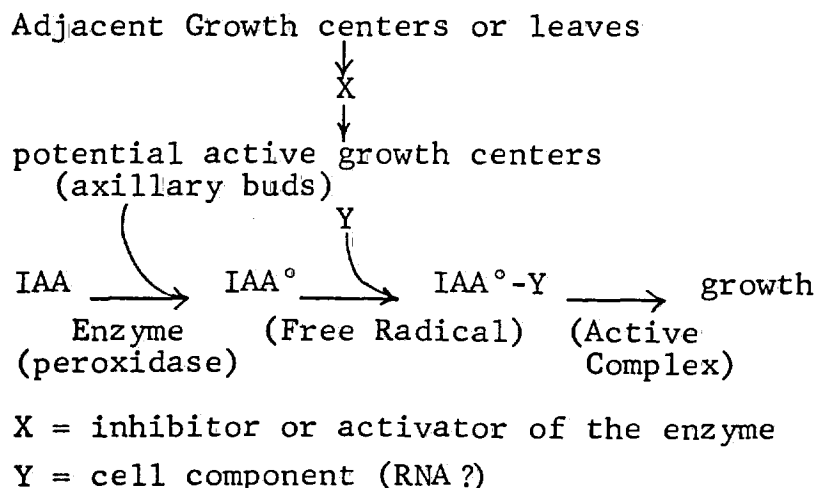
Plants treated with MH30 showed higher amino acid content of suckers, but also a different distribution of the various acids. It was concluded that MH30 interferes with sucker metabolism. It was noted by observation of the chromosomes that MH30 also interferes with mitosis.

ENZYMATIC OXIDATION OF INDOLE-3-ACETIC ACID, A PLANT GROWTH HORMONE, IN TOBACCO AND ITS POSSIBLE APPLICATION TO THE SUCKER CONTROL PROBLEM.

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ABSTRACT

This study of the metabolism of Indole-3-Acetic Acid (IAA) emphasizes its enzymatic oxidation by peroxidase enzymes. In accordance with a new concept of IAA action, the enzymatic oxidation of IAA in plants is considered an essential step in its action in promoting growth or cell-elongation in plants. Experimental evidence leads to the following formulation of the process of IAA transformation as it may occur in plants:

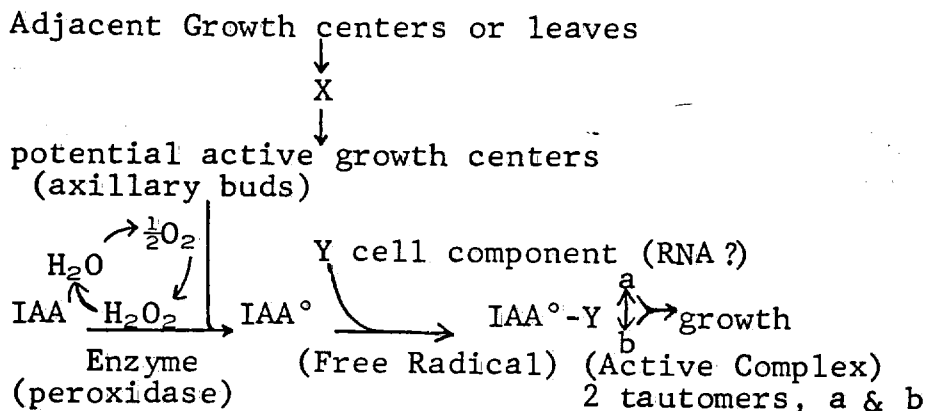


From the above postulate that IAA needs to be enzymatically transformed in order to be physiologically active, it is conceivable that by controlling any one of the steps leading to the formation of the active complex, one also controls the growth of suckers. Using a model system, Horseradish-peroxidase and cofactors, we found that various compounds known to inhibit growth also inhibited the oxidation of IAA. Several of these compounds such as 6-Aza-Uracil, Iodoacetate, and MH-30 will be discussed.

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REVIEW BY R. A. LUTZ

This study shows that there is an irreversible increase in cell-elongation, or growth in plants, due to the enzymatic oxidation of Indole-3-Acetic Acid (IAA) by peroxidase. This new concept is an essential step in cell-elongation, and experimental evidence presented leads to the following formulation of the process of IAA transformation as it may occur in plants:



The  $\text{H}_2\text{O}_2$  generating system includes dichlorophenone and  $\text{MnCl}_2$ . This concept indicates that Indole-3-Acetic Acid (IAA) has to be oxidized to the active form in order to promote elongation. Phenols interfere with the  $\text{H}_2\text{O}_2$  generating system and block the enzymatic oxidation of IAA.

From the above postulate that Indole-3-Acetic Acid (IAA) needs to be enzymatically transformed in order to be physiologically active, the author conceived that by controlling any one of the steps leading to the formation of the active complex, one also controls the growth of suckers.

To study this reaction, the author used a model system, Horseradish-peroxidase and cofactors. The active complex was separated by electrophoresis. Three compounds were isolated and found to be biologically active by a straight growth test.

The author noted that leaf growth was in relation to the activity of the dichlorophenone- $\text{MnCl}_2$  enzyme system and that two fractions contained peroxidase activity. One fraction was more dependent on  $\text{H}_2\text{O}_2$  and pH 6.1, the other more dependent on pH 6.8.

The effects of compounds on Indole-3-Acetic Acid activity and on cell growth were studied. There was a correlation between inhibition of growth and enzymatic activity. Mono-phenols such as hydroquinone, pyro-catechol, and 6-Aza-Uracil showed good control of sucker growth, while MH-30 (Maleic Hydrazide) had no effect.

The author concluded that various compounds known to inhibit growth also inhibit the oxidation of Indole-3-Acetic Acid.

EFFECTS OF CONSTITUENTS FROM  
INHIBITED BUDS UPON OXIDATION  
OF IAA AND THE GROWTH OF  
TISSUE CULTURES

G. W. Schaeffer, J. G. Buta,  
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tural Research Service, Crops  
Research Division, Plant  
Industry Station, Beltsville,  
Maryland

ABSTRACT

In the search for natural growth inhibitors, the axillary buds on non-topped plants seemed a likely source of materials with inhibitory products, albeit somewhat difficult to obtain. Quiescent axillary buds were extracted with methanol-H<sub>2</sub>O and the extracts were separated into ten components according to R<sub>f</sub> and ultraviolet fluorescence. The bud extracts were eluted from the paper and checked for the inhibition of IAA oxidation by the commercial enzyme, horse-radish peroxidase (HRP). Similar extracts were also checked for inhibitors of cell division with tissue cultures of N. suaveolens X langsдорiffii and assayed for growth-promoting activity with N. suaveolens. Generally speaking, the most fluorescent of the bands contained the most potent inhibitors of the HRP system. Cell division activity of the hybrid tissue cultures was not affected. Several bands promoted cell division with N. suaveolens.

REVIEW BY G. H. BURNETT

Axillary buds on non-topped tobacco plants were quick-frozen in liquid nitrogen, freeze-dried, and powdered. The powder was then extracted with 70% methanol-water. The alcohol extract was then paper chromatographed with an isopropanol: ammonia solvent. Ten components were separated.

These components were eluted from the paper and checked for inhibition of Indole-3-Acetic Acid (IAA) by the commercial enzyme, horse-radish peroxidase (HRP). Other samples of the extracts were also checked for inhibitors of cell division with tissue cultures of N. suaveolens X langsдорiffii and assayed for growth-promoting activity with N. suaveolens.

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Figure No. 1 shows the ultraviolet bands of the components and the two tobacco species. After band 4A there was no ultraviolet. Chlorogenic acid came off between 2 and 2A. Generally, the most fluorescent of the bands contained the most potent inhibitors of the HRP system.

Figure 1

Band Designation	Rf	Dilution Required for 1/2 Maximum Velocity of HRP	N. suaveolens	N. suaveolens X langsdoriffii
1	0.04	1-150	1.92	2.12
1A	0.10	1-175	2.16	2.76
2	0.17	1-150	2.28	2.64
2A	0.20	1-125	2.87	2.50
3	0.25	1-800	1.31	2.96
3A	0.32	1-1000	2.09	2.74
4	0.38	1-675	1.90	2.64
4A	0.45	1-1300	1.72	2.89
5	0.56	1-50	1.78	2.39
6	0.71	1-10	0.94	2.61
7	0.90	1-5	0.82	2.19
8	Paper only	1-5	1.08	2.49

In Figure No. 2 it is noted that there is quite an increase of growth in N. suaveolens at R<sub>f</sub> 0.2 and decrease in growth at R<sub>f</sub> .25. There is no significant difference in growth in the hybrid N. suaveolens X langsdoriffii. No correlation was found in peroxidase activity in this system.

TISSUE CULTURE STUDIES ON THE  
MECHANISMS OF ACTION OF MALEIC  
HYDRAZIDE AND 6-AZAURACIL

G. W. Schaeffer, USDA, Agricultural Research Service, Crops Research Division, Plant Industry Station, Beltsville, Maryland

ABSTRACT

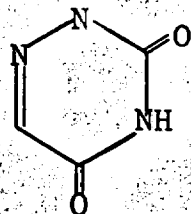
One of the problems encountered in studies concerned with the mechanism of action of specific compounds is the uptake and availability of each compound in question. One way to lessen the effects is to check the inhibition of growth in tissue culture. Cells of the interspecific hybrid N. SuSull grow rapidly on a defined agar medium and have served as a useful tool in the study of such growth inhibitors as azauracil and maleic hydrazide. Growth of tobacco cells can be completely inhibited by the addition of  $10^{-3}$ M azauracil to the agar medium. The inhibition is reversible with the addition of uracil or uridine to culture medium. Thus, it appears that the growth response to azauracil is similar in tobacco cells and bacterial cells. The inhibition of these cells by maleic hydrazide is not reversible with uracil and uridine. The mechanism of action is very different for these two inhibitors. The growth inhibition of azauracil and maleic hydrazide was observed with tissue of N. tabacum as well as with one of the parental types of the hybrid.

REVIEW BY H. C. SILBERMAN

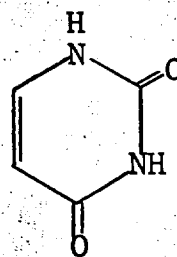
This paper is part of the symposium on the biochemical aspects of the growth and development of tobacco suckers. It deals with the drying out of known growth inhibitors.

Azauracil is an anticancer drug reported by Czechoslovakian researchers. The formulae of the inhibitors used are given below:

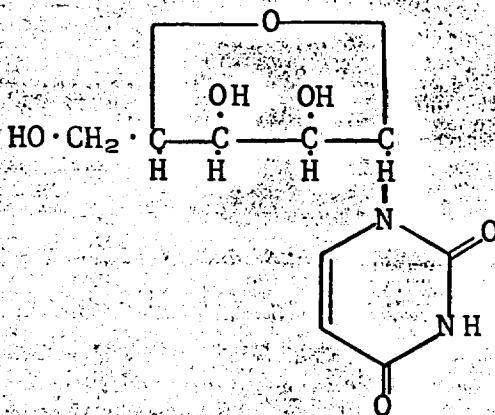
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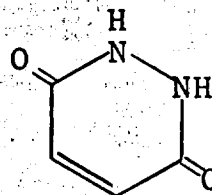
6-azauracil



uracil  
(2,4-dioxypyrimidine)

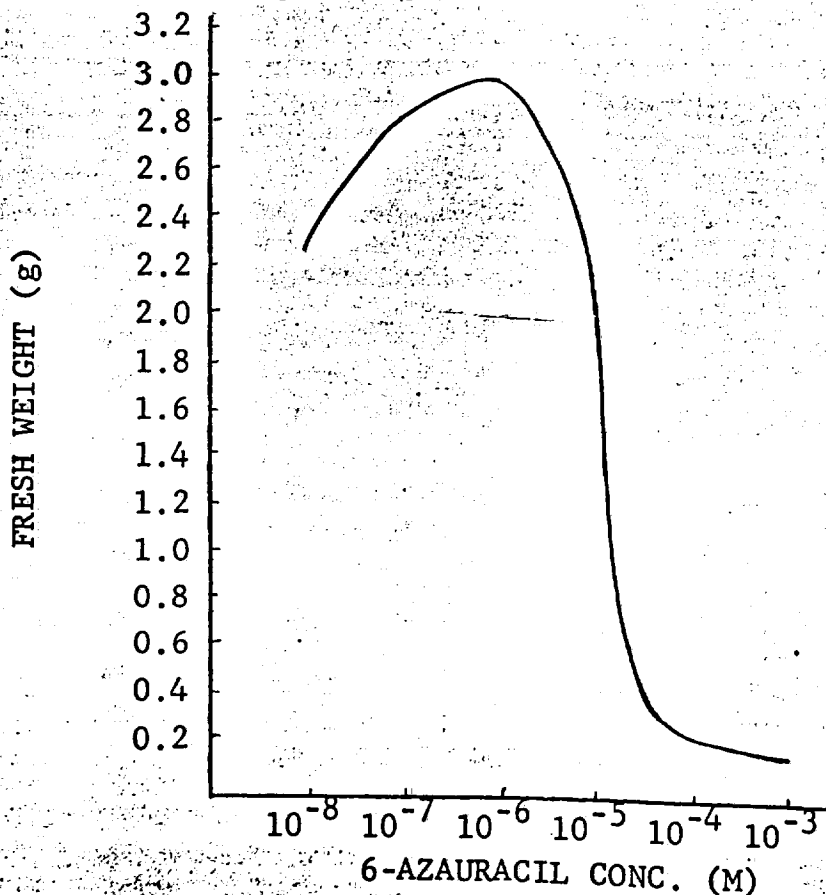


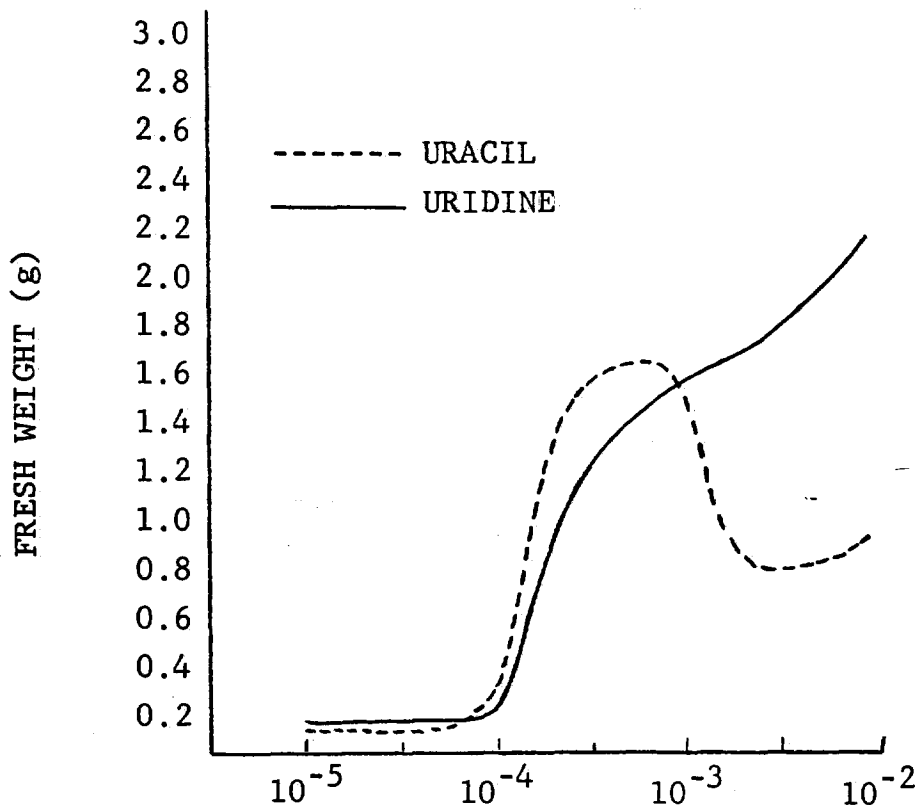
uridine



maleic hydrazide

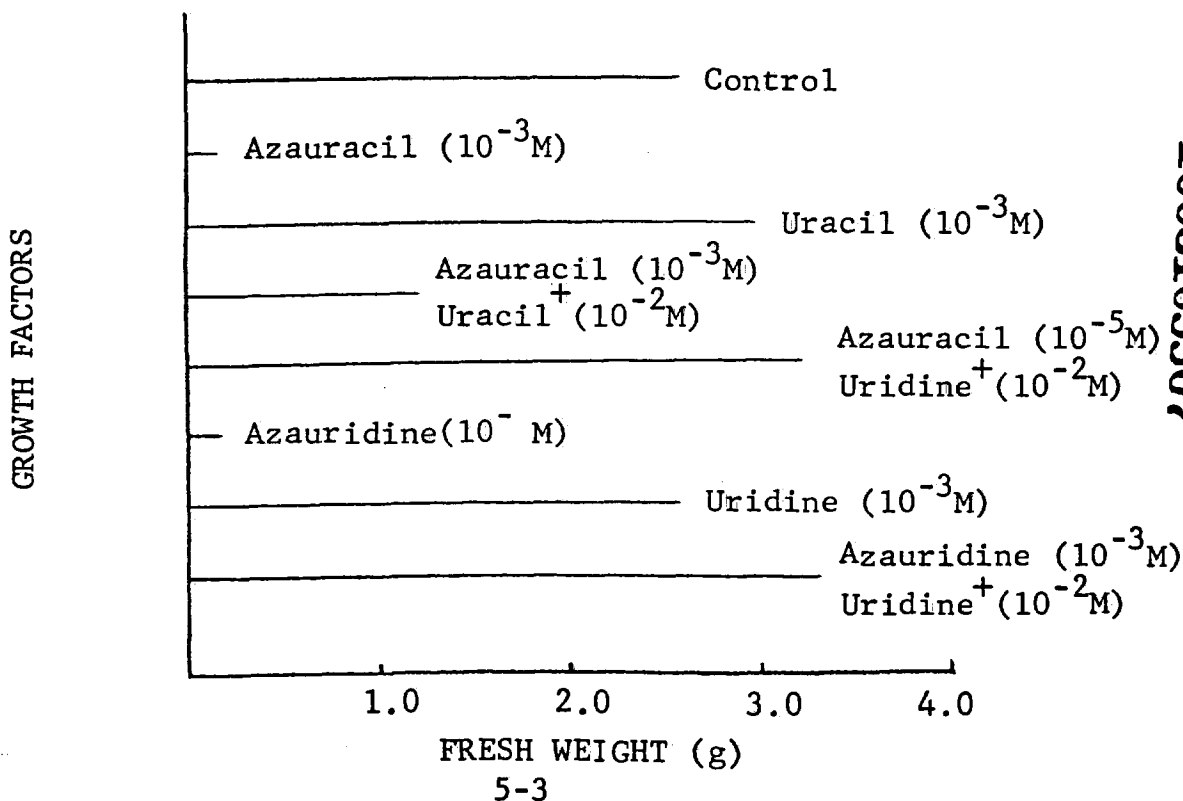
The effect of azauracil, uracil, and uridine alone was shown graphically:



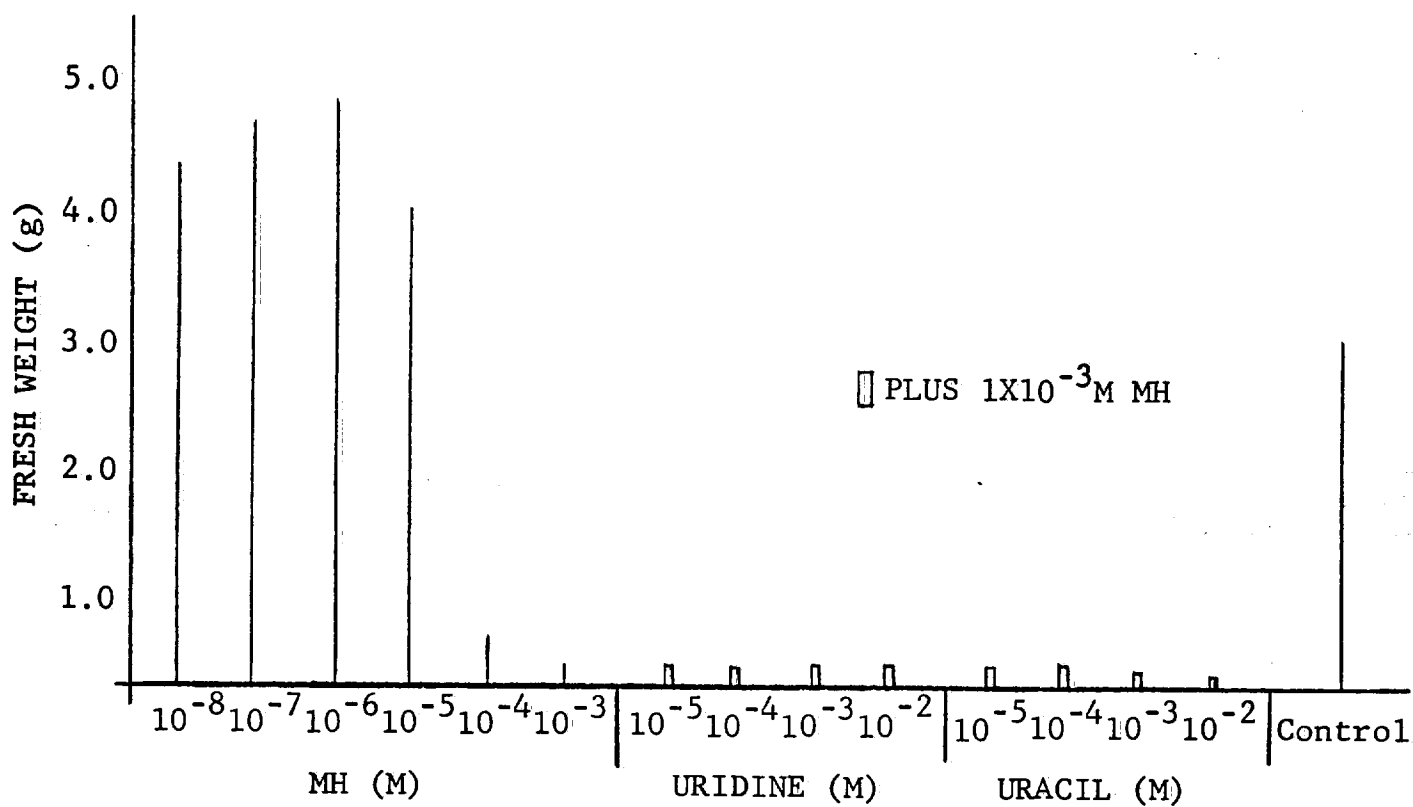


Azauracil, for instance, promotes the growth of tobacco cells at concentrations up to  $10^{-6}$  M. At higher concentrations there is complete inhibition of growth.

The effect of combinations of plant inhibitors was also shown graphically (MH stands for maleic hydrazide):



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THE INHIBITION OF TOBACCO AXILLARY  
BUD GROWTH WITH FATTY ACID DERIVA-  
TIVES

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USDA, Agricultural Research Ser-  
vice, Crops Research Division,  
Plant Industry Station, Beltsville,  
Maryland

ABSTRACT

Methyl esters of fatty acids with chain lengths from  $C_6$  to  $C_{18}$  have been shown to cause inhibition of tobacco axillary bud (sucker) growth. The fatty acid derivatives are applied as a water emulsion with a wetting agent and, to be effective, must come in direct contact with the young axillary buds. At proper concentration levels and as part of a suitable emulsification system, these materials caused damage to the young meristematic and differentiating tissue, but tissues in more advanced stages of maturity were not affected. Results with 4 tobacco types under field conditions show that the methyl esters of fatty acids with 8 to 14 carbon atoms gave a high degree of inhibition. Generally, the methyl ester of the  $C_{10}$  acid was the most effective. Fatty alcohols were also found to be effective for the control of sucker growth. Fatty alcohols with chain lengths from  $C_8$  to  $C_{12}$  were tested on field grown tobacco and the  $C_{10}$  material again appeared to be the most effective. The physical properties of fatty alcohols with chain lengths longer than 12 prevent them from being useful for this purpose. A number of fatty acid derivatives other than the methyl esters and fatty alcohols have also been shown to be effective. In certain types of tobacco, plants are allowed to remain standing for several weeks after treatment. Secondary suckers may begin to develop under this condition. A second application of the fatty acid derivative will therefore be required. If low amounts of certain systemic growth regulators are mixed with the fatty acid derivatives, the combination effectively controls the growth of both primary and secondary suckers.

This paper was a review of the following articles, which are available in the library:

"Plant-growth Inhibition by some Fatty Acids and their Analogues," Tso, T.C., Nature, 202, #4931, 511-512 (May 2, 1964)

"Preliminary Observations on Inhibition of Tobacco Suckers by Vegetable Oils and Fatty Acids," Tso, T.C., and McMurtrey, J.E., Jr., Tobacco Science, 7, 101-104 (June 7, 1963)

"Inhibition of Tobacco Axillary Bud Growth with Fatty Acid Methyl Esters," Tso, T.C., Steffens, G.L., and Engelhaupt, M.E., Agricultural and Food Chemistry, 13, #1, 78 (Jan./Feb. 1965)

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PRELIMINARY OBSERVATIONS ON THE  
MECHANISM OF SUCKER INHIBITION  
WITH METHYL LAURATE

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Research Service, Crops Research  
Division, Plant Industry Station,  
Beltsville, Maryland

ABSTRACT

C-14 labeled methyl laurate was applied to decapitated tobacco plants to study the possible translocation of these materials as sucker control agents. Five areas of application were used. C-14 methyl laurate was applied as follows: (1) painted around the 2-inch area of the tip of the decapitated plant; (2) sprayed on the surface of three upper leaves; (3) painted on all axillary buds of the plant; (4) painted on the upper part of the root adjacent to the crown area; and (5) sprayed on the tobacco plant as usually applied in the field. Twenty-four hours after treatment plants were harvested. The plant parts which were treated with methyl laurate were first washed with ethanol and then extractions were made to examine the presence of C-14 labeled material. The remainder of the plant was divided into upper, middle, and bottom parts which were extracted with ethanol to permit a study of the distribution of radioactivity, if any. Results indicated that C-14 is localized in the area where the treatment was applied. Little or no activity was detected in any other area of the plant. It appears that methyl laurate inhibited sucker growth by contact only.

REVIEW BY H. D. MERWIN

Methyl laurate is used in sucker control by spraying it on to the tobacco plant. This study was undertaken to find out if methyl laurate, a foreign molecule, was subject to reactions whose products might affect the physiology of the plant. Among other questions two important ones were: (1) Does translocation occur? and (2) Does metabolism take place?

The laurate part of the molecule was furnished by C-14 labeled lauric acid. The methyl laurate into which it was incorporated was applied to five parts of decapitated tobacco plants, as described in the abstract. 13.6% of the methyl laurate applied to the surface of the plant was recovered from the surface. Of that which presumably penetrated the axillary buds, 20% was recovered at the site; and of that which presumably penetrated the top of the roots, 10% was recovered from the area of application. Very little was found in any part of the plant away from the site

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of application. This is evidence that there was no translocation of methyl laurate. The fate of the unrecovered C-14 is unknown. Furthermore, the ratio of C-14 to other carbon was measured several times before and after application of the C-14 methyl laurate. There were no changes, which led to the conclusion that methyl laurate is not metabolized.

The general conclusion is that methyl laurate acts by contact only and the actual mechanism is unknown.

POSSIBLE APPROACHES TO THE BIO-CHEMICAL ALTERATION OF SUCKER GROWTH AND LEAF CONSTITUENTS WITH NUCLEIC ACID COMPONENTS AND ANALOGS

G. W. Schaeffer and G. L. Steffens, USDA, Agricultural Research Service, Crops Research Division, Plant Industry Station, Beltsville, Maryland

ABSTRACT

Numerous growth and developmental phenomena are controlled directly and indirectly by changes in the nucleic acid constituents of the cell. The generalized scheme of information flow is from DNA  $\xrightarrow{\hspace{1cm}}$  RNA  $\xrightarrow{\hspace{1cm}}$  proteins. The information flow can be intercepted or altered with natural or environmental changes, or chemically altered with growth factor analogs. Some growth factor analogs which specifically inhibit RNA synthesis in plant, animal and bacterial cells are azauracil and its riboside, azauridine. Azauracil inhibits sucker growth of N. Xanthine when applied at  $3 \times 10^{-5}$  M solution. The inhibition can be reversed with high levels of uridine which suggests that tobacco growth can be modified to a limited extent with the addition of exogenous growth factors. Greenhouse studies with both flue-cured and burley tobacco types have shown that azauracil causes some inhibition of sucker growth. However, azauracil at concentrations high enough to inhibit sucker growth, causes leaf injury. When uracil and/or uridine is applied with azauracil, leaf injury is reduced. Spray solutions containing low levels of maleic hydrazide and azauracil, along with uracil and/or uridine, gave a high degree of sucker control without visible changes in the leaf. We conducted field experiments with both flue-cured and burley tobacco to determine whether the addition of azauracil, uracil and/or uridine, along with low levels of maleic hydrazide, would effectively control sucker growth and whether they would alter the chemical and physical properties of cured leaf.

REVIEW BY G. H. BURNETT

The authors attempted in this paper to show how chemical suckering agents interrupt or alter the natural information flow of DNA  $\xrightarrow{\hspace{1cm}}$  RNA  $\xrightarrow{\hspace{1cm}}$  proteins. The authors used the scheme of DNA  $\xrightarrow{\hspace{1cm}}$  plus ribosomes  $\xrightarrow{\hspace{1cm}}$  polysomes, after the work of Calvin.

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Some of the agents may prevent DNA flow but not RNA, and some may prohibit proteins but allow RNA. The chemical agent azauracil probably inhibits RNA and loss of transfer RNA and proteins not formed.

Table No. 1 shows the inhibition of growth due to several chemicals. Azauracil alone and in combination with uracil showed good inhibition, whereas uracil alone did not.

Table 1

Effects of Nucleic Acid Analogs and Normal Constituents Upon the Growth of Axillary Buds (suckers) in Tobacco Plants

<u>Treatment</u>	<u>Conc. (M)</u>	<u>Number of Plants</u>	<u>% Inhibition<sup>1</sup></u>
Azauracil (sp)	$3 \times 10^{-3}$	32	85
Azauracil (p)	$3 \times 10^{-3}$	8	99
Uracil (sp)	$1 \times 10^{-2}$	8	9
Azauracil (sp)	$3 \times 10^{-3}$		
+ uracil (sp)	$1 \times 10^{-2}$	8	95
Azauracil (sp)	$3 \times 10^{-3}$		
+ uradine (sp)	$1 \times 10^{-1}$	12	71
+ uradine (sp)	$6 \times 10^{-1}$	4	8
Azauradine (sp)	$1 \times 10^{-3}$	4	62
Azauradine (sp)	$1 \times 10^{-3}$		
+ uradine (sp)	$6 \times 10^{-1}$	4	-8*
Diazouracil (p)	$3 \times 10^{-3}$	8	98
Diazouracil (sp)	$3 \times 10^{-3}$	4**	0

\* More bud growth than with control

\*\* An additional experiment with Connecticut Broadleaf also gave no inhibition

(sp) Growth factor applied as a spray

(p) Growth factor applied as a lanolin paste

<sup>1</sup> % Inhibition refers to:  $\frac{\text{Control minus treated}}{\text{control}} \times 100$

Tests were made by three methods: 1) greenhouse plants, 2) roots-water system and 3) field trials. Azauracil applied at the root system was translocated and gave leaf damage.

Results of greenhouse experiments with four sucker inhibitors are shown in Table No. 2. Maleic hydrazide at 50 mg gave complete inhibition, but at 10 mg it gave about one-half inhibition.

Table 2  
Greenhouse Experiments with Combinations  
of MH, Azauracil, Uracil and Uridine<sup>1</sup>

Treatment <sup>2</sup>	Burley	% Sucker Control	
		Conn. Brdlf. (A)	Conn. Brdlf (B)
1. TNS	0	0	0
2. MH (50 mg)	100	100	96
3. MH (10 mg)	34	59	53
4. Azauracil (10 mg)	59	21	31
5. MH (10 mg) + Azauracil (10 mg)	93	83	86
6. MH (10 mg) + Azauracil (10 mg) + Uracil (40 mg)	98	87	-
7. Azauracil (10 mg) + Uracil (40 mg)	57	42	-
8. MH (10 mg) + Azauracil (10 mg) + Uridine (100 mg)	-	-	88
9. MH (10 mg) + Uracil (50 mg)	-	-	73

<sup>1</sup> 4 plants per treatment - 7 inch pots

<sup>2</sup> mg active material in 20 ml solution per plant

The following two tables show the results of sucker control in field experiments. In this experiment, 50 mg of maleic hydrazide which gave complete inhibition in greenhouse trials gave only about 50% inhibition in the field. The addition of 20 mg of azauracil to 50 mg of maleic hydrazide gave good control in burley, but only one quarter in flue cured.

Table 3

Degree of Sucker Control with Combinations  
of MH, Azauracil, Uracil and Uridine

<u>Treatment<sup>1</sup></u>	<u>% Sucker Control</u>	
	<u>Burley<sup>2</sup></u>	<u>Flue Cured<sup>3</sup></u>
1. Hand-Suckered	-	-
2. MH (170 mg)	98	84
3. MH (170 mg) + Uracil (60 mg) + Uridine (40 mg)	99	51
4. MH (50 mg)	51	-
5. MH (50 mg) + Azauracil (20 mg)	93	26
6. MH (50 mg) + Azauracil (20 mg) + Uracil (80 mg)	81	30
7. MH (50 mg) + Azauracil (20 mg) + Uracil (60 mg) + Uridine (40 mg)	75	49
8. Azauracil (20 mg) + Uracil (80 mg)	51	-

<sup>1</sup> Mg of active material in 20 ml solution per plant

<sup>2</sup> Produced at Mountain Experiment Station, Waynesville, N.C. 1965

<sup>3</sup> Produced at Pee Dee Experiment Station, Florence, S.C. 1965

The last table shows the effects of the suckering agent on chemical and physical properties of flue-cured tobacco.

Table 4

Chemical and Physical Properties of Flue-Cured Tobacco  
Treated with Combinations of MH, Azauracil, Uracil and Uridine<sup>1</sup>

Treatment <sup>2</sup>	Total Nitrogen %	Protein N Soluble %	Total Alkaloids %	Reducing Sugars %	Specific Volume cc/.33g	Equilib. Moisture %
Hand-Suckered	2.66	66.4	3.38	18.2	1.12	12.7
MH (170 mg)	2.38	63.5	2.87	22.5	1.05	13.4
MH (170 mg) + Uracil (60 mg)+ Uridine (40 mg)	2.29	62.2	2.40	22.7	1.03	13.2
MH (50 mg) + Azauracil (20 mg)	2.28	64.2	2.47	22.0	1.06	12.9
MH (50 mg) + Azauracil (20 mg) + Uracil (80 mg)	2.34	63.8	2.44	22.1	1.02	13.1
MH (50 mg) + Azauracil (20 mg) + Uracil (60 mg) + Uridine (40 mg)	2.18	62.5	2.66	21.7	1.07	13.0

<sup>1</sup> Produced at Pee Dee Experiment Station, Florence, S.C. 1965,  
average of Mid and Top third

<sup>2</sup> Mg active material in 20 ml solution per plant

1003109916

THE EFFECTS OF RIPENESS AND CURING  
RATES ON THE FREE AMINO ACIDS OF  
FLUE-CURED TOBACCO

J. A. Weybrew, J. M. Carr, and  
W. G. Woltz, North Carolina State  
of the University of North Carolina,  
Raleigh, North Carolina

ABSTRACT

Cured-leaf samples of Coker 319 that had been harvested at one of three stages of ripeness and subsequently cured either "fast" or "normally" or "slowly" with respect to yellowing time were analyzed for 29 "free" amino acids (defined on the basis of extractability with 1/100 HCl) or other ninhydrin-positive substances. Rather large differences, approaching 40-fold for certain constituents, were observed. Of the imposed variables, the differences associated with degree of ripeness were larger and more consistent; 27 constituents decreased with advancing ripeness (24 significantly) while only 2 showed no change. During curing, 16 constituents decreased (7 significantly) with prolonged yellowing while 11 increased (7 significantly). With regard to stalk position, 12 of the amino acids were higher in bottom primings than in the top but lowest in the middle of the stalk; twelve other constituents increased linearly up the stalk. Relative prevalences will be discussed from the viewpoints of metabolism, protein synthesis and proteolysis.

REVIEW BY E. J. DESZYCK

The picking times of the leaf samples studied were estimated by leaf color, being a week early for the unripe leaf, at optimum picking time and one week past maturity. The leaf quality was maximum at optimum time of harvest. The curing times were 3/4 time of normal, normal, and 5/4 time of normal for fast cure. The unripe fast cured leaf still had green color.

The effects of curing schedules and ripeness on the free amino acids are shown in the four tables. Results for aspartic acid are found in Table 1.

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Table 1

Overall Treatment Effects on Aspartic Acid ( $\mu\text{g}$  per gm)

		Curing Schedule			Ripeness Means
		<u>Fast</u>	<u>Normal</u>	<u>Slow</u>	
R I P E N E S S	Under-Ripe	539	551	645	579
	Ripe	341	338	414	364
	Over-Ripe	225	247	273	249
	Curing Means	369	379	444	

---

Position Means:	Top	312	Constituents Responding Similarly: Lysine, Histidine, Ammonia, STAG <sup>1</sup> , Glutamic Acid, Glycine		
	Middle	281			
	Bottom	598			

<sup>1</sup>serine, tryptophane, alanine, and glutamine

With ripeness, an inverse relationship was apparent, since the unripe leaf had the highest and the over-ripe the lowest amounts of this acid. With curing, a linear relationship is apparent. Most aspartic acid is present in the slow cure and least in the fast cure. The bottom leaves of the stalk were highest and the top leaves lowest in the amino acids listed in Table 1.

In general, the effect of ripeness on  $\alpha$ -amino adipic acid, proline, and their associated amino acids follows the same trend as does the effect of ripeness on valine (Tables 2, 3, 4). With curing, proline follows the same trend as does aspartic acid. However, for both  $\alpha$ -amino adipic acid and valine the trend is reversed, being highest for the fast cure and lowest for the slow cure (Tables 2, 3). The valine relationship, in regard to position of the leaves on the stalk, is similar to that for aspartic acid, but for  $\alpha$ -amino adipic acid and proline the trend is reversed, the highest levels being found in the top leaves and the lowest in the bottom leaves (Tables 3, 4).

Eventually, the tobaccos of various curing times, degrees of ripeness, and stalk positions will be evaluated as to smoke flavor.

Table 2

Overall Treatment Effects on Valine ( $\mu\text{g}$  per gm)

		<u>Curing Schedule</u>			<u>Ripeness Means</u>
		<u>Fast</u>	<u>Normal</u>	<u>Slow</u>	
Ripeness	Under-Ripe	266	216	194	225
	Ripe	167	152	134	151
	Over-Ripe	135	81.4	91.4	102
	Curing Means	189	150	140	

Position Means:	Top	84.7	Similar Response From: Arginine, Cystine, Isoleucine, Leucine, Phenylalanine <sup>+</sup> , $\gamma$ -Aminobutyric Acid		
	Middle	97.4			
	Bottom	296			

Table 3

Overall Treatment Effects on  $\alpha$ -Aminoadipic Acid (ug per gm)

		<u>Curing Schedule</u>			<u>Ripeness Means</u>
		<u>Fast</u>	<u>Normal</u>	<u>Slow</u>	
Ripeness	Under-Ripe	308	315	259	294
	Ripe	276	287	201	255
	Over-Ripe	231	238	231	233
	Curing Means	272	280	230	

Position Means:	Top	324	Similar Response From: L <sub>3</sub> , L <sub>4</sub> , L <sub>5</sub> , Alanine, Tyrosine, Homocystine, Galactosamine, L <sub>11</sub> , L <sub>12</sub>		
	Middle	293			
	Bottom	165			

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Table 4

Overall Treatment Effects on Proline (ug per gm)

		<u>Curing Schedule</u>			<u>Ripeness Means</u>
		<u>Fast</u>	<u>Normal</u>	<u>Slow</u>	
Ripeness	Under-Ripe	4870	5910	6390	5720
	Ripe	3430	4340	4950	4240
	Over-Ripe	2090	2960	3680	2910
	Curing Means	3460	4400	5010	

Position Means: Top 7930  
Middle 2640  
Bottom 2310

Similar Response From:  
Hydroxyproline

MICROBIAL DEGRADATION OF NICOTINE  
II - EFFECT OF ENVIRONMENTAL CON-  
DITIONS AND COMPARISON OF VARIOUS  
AVAILABLE NICOTINE DEGRADING BAC-  
TERIAL ISOLATES

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General Cigar Company, Inc.,  
Lancaster, Pennsylvania

ABSTRACT

Under improved conditions, nicotine-degrading bacterial isolates which did not form blue pigment tolerated and metabolized more nicotine. These conditions consisted of the use of a high-speed rotary shaker and Erlenmyer flasks modified by forming two elongated indentations in opposite walls of the flask to function as baffles. Nicotine-degrading bacterial isolates obtained from various investigators were compared with our own cultures using conventional biochemical tests. Freeze-dried cultures grown in two different media also were compared by an infra-red spectrophotometric technique. Oxidation of various compounds by the previously reported six nicotine-degrading isolates was investigated using a manometric technique. Bacterial isolates which did not form blue pigment in nicotine-containing medium lost their ability to oxidize nicotine after being lyophilized. The same isolates, freshly harvested, oxidized nicotine at a faster rate than those which formed blue pigment. When the isolates were grown in a medium containing conventional sources of carbon and nitrogen instead of nicotine, only one oxidized nicotine to a small extent. However, all isolates oxidized nicotine readily when grown in a conventional medium supplemented with nicotine.

REVIEW BY A. B. CLARKE

This was a continuation of work reported last year on the microbial degradation of nicotine. Certain improvements were made to oxidize nicotine more readily. A high speed shaker and a modified Erlenmyer flask increased aeration to step up the degradation of nicotine. Cultures obtained from various investigators were compared using the improved technique. (Table 1)

1003109921

TABLE 1

## CULTURAL CHARACTERISTICS OF BLUE PIGMENT-FORMING, NICOTINE-DEGRADING BACTERIAL ISOLATES

Isolate	#1	#4	P-34	P.S.	KD	LiC	Cer
Source	Soil	Tobacco Seed	Rittenberg	Sgueros	Decker	Stone	Stone
Morphology (Nicotine Medium)	-----Short Rods, Some Coccoid Forms-----						
Size ( $\mu$ )	-Coccoid Forms 0.4 x 0.5, Short Rods 0.5 x 0.7 to 0.6 x 0.8-----						
Color:							
Nutrient Agar	Off-White	Off-White	Yellow	Pale Yellow	Pale Yellow	Off-White	Off-White
Nicotine Medium	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Gram Reaction	-	-	-	-	-	-	-
Nitrate Reduction	+	+	-	+(weak)	+	+	-
Litmus Milk (pH/Digestion)	Acid/-	Alkaline/+	Alkaline/-	Alkaline/-	Alkaline/+	Alkaline/+	Alkaline/
Casein Hydrolysis	+	+	+	-	+	+	-
Gelatin Liquefaction	Rapid	Rapid	-	Slow	Rapid	Slow	Slow
Starch Hydrolysis	-	-	-	-	+	-	-
H <sub>2</sub> S-Formation	+	+	-	-	-	+	-
Indol Formation	-	-	-	-	-	-	-
Acetylmethylcarbinol	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Nicotinic Acid	-	-	-	-	+	-	-
Glucose, Sucrose Fructose (growth/pH)	+/-Acid	+/-Alkaline	+/-Alkaline	+/-Alkaline	+/-Alkaline	+/-Alkaline	+/-Alkaline

Oxidation of nicotine-related compounds grown in Sgueros medium are shown in Table 2. Those bacterial isolates which lyophilized did not form blue pigment and did not oxidize nicotine. However, if the isolates are fresh, those forming blue pigment are slower in oxidizing nicotine than are those forming another color.

TABLE 2

OXIDATION OF NICOTINE-RELATED COMPOUNDS  
BY WASHED SUSPENSIONS OF ISOLATES NOS. 1-6  
GROWN IN SGUROS' MEDIUM

Substrate (0.02 M)	- $\Delta O_2$ ( $\mu$ l/mg. dry cell wt./2 hrs.)					
	Isolate Numbers					
	1	2	3	4	5	6
Pseudo-Oxynicotine	15.7	198.5	264.5	20.2	16.2	290.0
Oxynicotine	24.1	42.5	26.1	11.7	30.2	62.9
Myosmine	22.0	17.4	31.0	26.3	38.0	15.7
Nicotyrine	-	-	-	-	-	-
Nicotine-Ethylene Oxide Complex	-	-	-	-	-	-

The oxidation of nicotine grown in different media is shown in Table 3. Notice that nicotine is oxidized in Sgueros' Medium. Nutrient Broth is not effective unless supplemented with nicotine.

TABLE 3

THE OXIDATION OF NICOTINE (0.02 M)  
BY WASHED SUSPENSIONS OF BACTERIAL ISOLATES NOS. 1-6  
GROWN IN DIFFERENT MEDIA

	- $\Delta O_2$ ( $\mu$ l/mg. dry cell wt./2 hrs.)					
	Isolate Numbers					
	1	2	3	4	5	6
Sgueros' Medium	53.1	231.5	294.5	65.5	263.5	193.5
Nutrient Broth	-	-	27.8	-	-	-
Nutrient Broth + Nicotine	28.9	48.2	203.5	50.4	165.0	163.5
Nutrient Broth followed by 5 hours in Nicotine	-	-	23.0	-	-	-

Paper No. 11

THE ENZYMATIC DEGRADATION OF THE  
STRUCTURAL CARBOHYDRATES IN  
SELECTED TOBACCOS.

Henri C. Silberman, Philip Morris  
Inc., Richmond, Virginia.

ABSTRACT

Cured bright and burley laminas and stems, green tobacco, tobacco pretreated with acid, and tobacco treated with ultrasonic waves were subjected to the action of cellulase, hemicellulase, and pectinase present in commercial enzyme preparations. The extent of the reaction was determined by chemical analysis, by microscopic observation, by paper chromatography, and by the weight-loss of the plant material after enzymatic attack. Photomicrographs of enzyme-treated tobacco indicate that the thin parenchymatous cell walls are readily broken down. Some, but not all, enzyme preparations produce reducing sugars as they act on tobacco material. The amounts of cellulose, hemicellulose, and pectin solubilized by enzyme preparations are shown to be a function of enzyme concentration, reaction time, enzyme specificity, and substrate accessibility. The values obtained from the action of the same enzyme preparations on purified polysaccharides are discussed to show correlations with the results obtained on the whole plant. Ultrasonic treatment and pretreatment with acid enhance the action of enzymes. Polysaccharides in cured bright tobacco are more easily solubilized than polysaccharides in cured burley tobacco. The fate of tobacco alkaloids during the enzyme treatment is discussed.

A copy of this paper is available in the library.

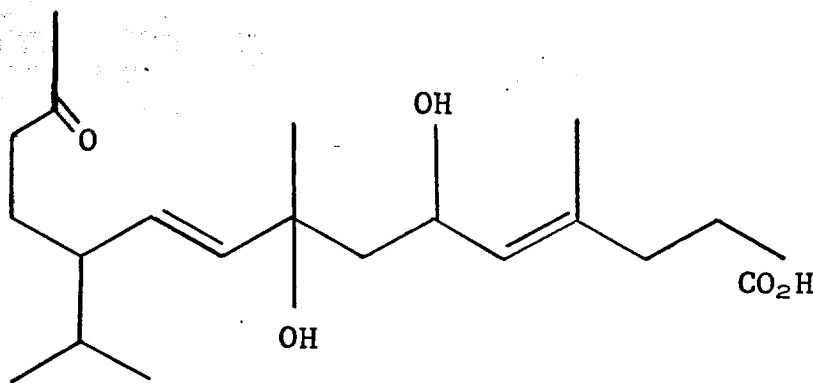
1003109924

STRUCTURE OF TWO SOLANONE PRE-  
CURSORS FROM TOBACCO.

G. W. Kinzer, Battelle Memorial  
Institute, Columbus, Ohio, and  
R. R. Johnson, Brown and William-  
son Tobacco Corporation, Louis-  
ville, Kentucky.

ABSTRACT

Two carboxylic acid precursors of solanone (2-methyl-5-isopropyl-1, 3-nonadien-8-one) have been isolated from a chloroform extract of burley tobacco using preparative TLC of the methyl esters for final purification. Both precursors give solanone in 7.5% weight yield on gas chromatography assay. The methyl esters of both precursors give (1) methyl levulinate and 2-isopropyl-5-keto-hexanal on ozonolysis in the presence of tetracyanoethylene, (2) a single saturated keto ester with mass 340 on forced hydrogenation (methyl 4, 8-dimethyl-11-isopropyl-14-keto-pentadecanoate), and (3)  $\alpha$ ,  $\beta$ -unsaturated ketones from chromium trioxide-pyridine oxidation which undergo reverse aldolization to form norsolanadione (5-isopropyl-3-nonene-2,8-dione) and 4-methyl-6-ketoheptanoic acid. Further structural data on the precursors from the infrared and NMR spectra, and their thermal decomposition to give solanone allows only diastereoisomers of 4,8-dimethyl-6,8-dihydroxy-11-isopropyl-14-keto-4,9-pentadecadienoic acid (Structure I) as possible structures.



This paper is published in The Journal of Organic Chemistry 30 (9), 2918-2921 (1965). The work is covered in U. S. Patent 3,174,485, March 23, 1965.

1003109925

AN INSTRUMENT FOR MEASURING CUT  
TOBACCO MOISTURE CONTENT BY MEANS  
OF MICROWAVE ABSORPTION.

J. O. Pullman, W. J. Hollenbeck,  
and G. W. Gibson, Research  
Department, Liggett and Myers  
Tobacco Company, Durham, North  
Carolina.

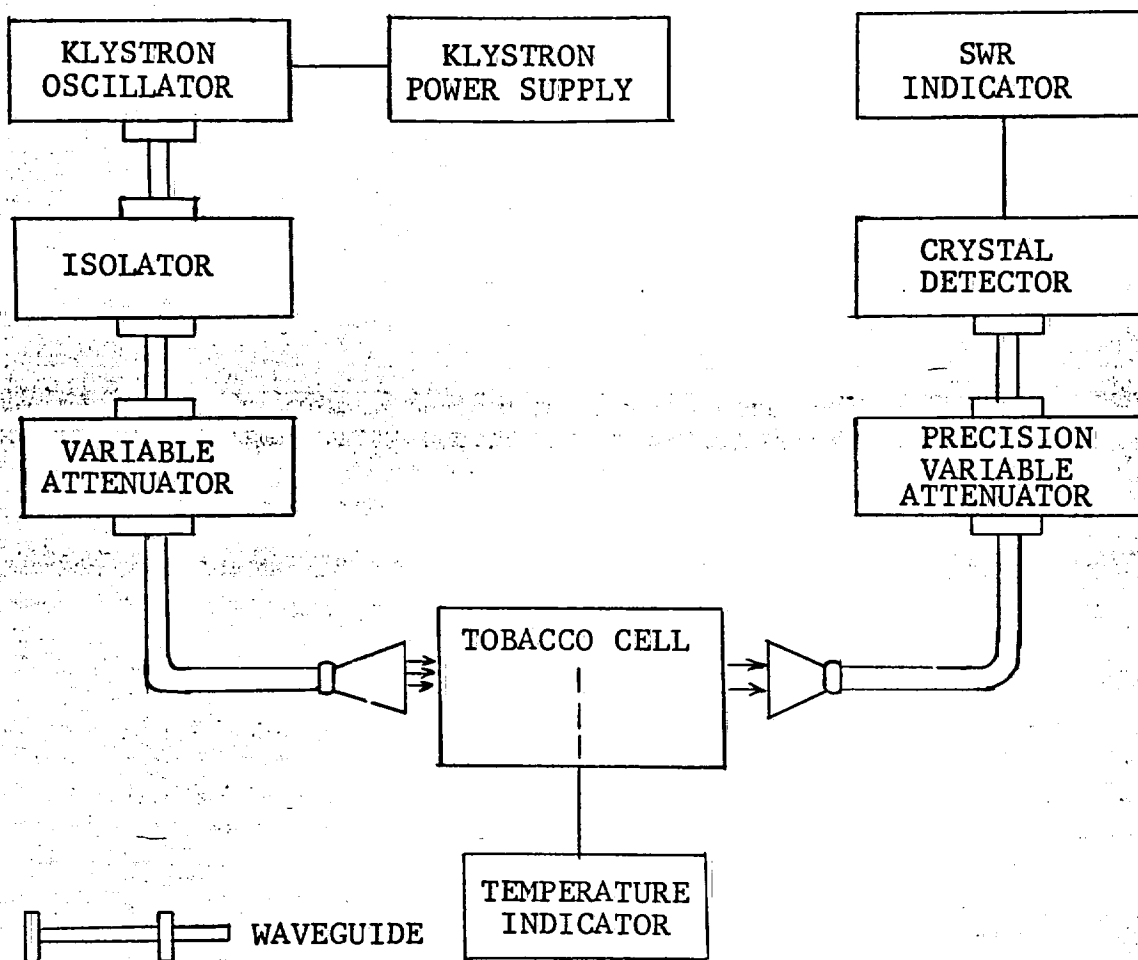
ABSTRACT

In the production of tobacco for smoking purposes, moisture content is an important variable which has an optimum value at each point in the process. An accurate means of measurement is therefore necessary. Desiccation in an oven or with drying agents gives good results but is too slow for production use. A variety of instruments is available that measures some property of tobacco, usually an electrical one, which is itself sensitive to moisture. When calibrated against a standard, such instruments will give estimates of the moisture content of a tobacco sample. These devices have been widely used, and with varying degrees of success. This paper describes a batch-type instrument for measuring the moisture content of cut tobacco by means of microwave absorption, a technique which offers unique advantages. The instrument features a removable tobacco holder suitable for one-pound samples, and may easily be operated by unskilled personnel. The instrument responds to the total amount of water present, and the absorbed power, expressed in decibels, varies linearly with percent moisture over a wide moisture range. In repeated measurements on identical samples standard deviations of between 0.25 and 0.10% H<sub>2</sub>O are obtained.

REVIEW BY A. B. CLARKE

This paper described an instrument for measuring moisture in tobacco by means of microwave absorption. Calibration was obtained with tobacco filler stored 9 days over anhydrous sulfuric acid (in a desiccator). Repeated measurements using the instrument showed standard deviations between 0.10 and 0.25% moisture. Following is a box diagram of the measuring unit.

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The laboratory equipment is commercially available. Liggett and Myers presently is using three units in their factories on a routine basis. The instruments are rapid and do not require skilled personnel for their operation.

Several unique features of the cell (tobacco holding device) are the following: 1. It is circular to prevent dead spots and is constructed of metal to prevent loss of moisture. 2. Its length is greater than its diameter to give better results in non-homogeneous samples. 3. A temperature measuring probe is injected into the sample at the bottom when the cell is placed in position on the factory model. Corrections for temperature are made directly on the instrument by a dial setting.

Tobacco is packed into the cell on a vibrating table to assure uniform distribution. The cell holds about 450 grams of tobacco. Different blends of tobacco may vary slightly in indicated moisture content. It is suggested that in such cases a calibration curve be prepared.

A patent application has been filed but the patent has not issued. The application covers the configuration but not the principle of the instrument.

Paper No. 14

DIRECT VAPOR CHROMATOGRAPHIC  
DETERMINATION OF MENTHOL, PRO-  
PYLENE GLYCOL, NICOTINE, AND  
TRIACETIN IN CIGARETTE SMOKE.

Larry A. Lyerly, R. J. Reynolds  
Tobacco Company, Winston-Salem,  
North Carolina.

ABSTRACT

A simple method which requires no separation techniques is presented for the determination of menthol, propylene glycol, nicotine, and triacetin in cigarette smoke. The procedure consists of smoking onto a Cambridge filter which quantitatively collects the particulate phase, and the smoke components are then distilled directly from the filter in a stream of helium onto a chromatographic column for analysis. All four components are determined from a single chromatogram.

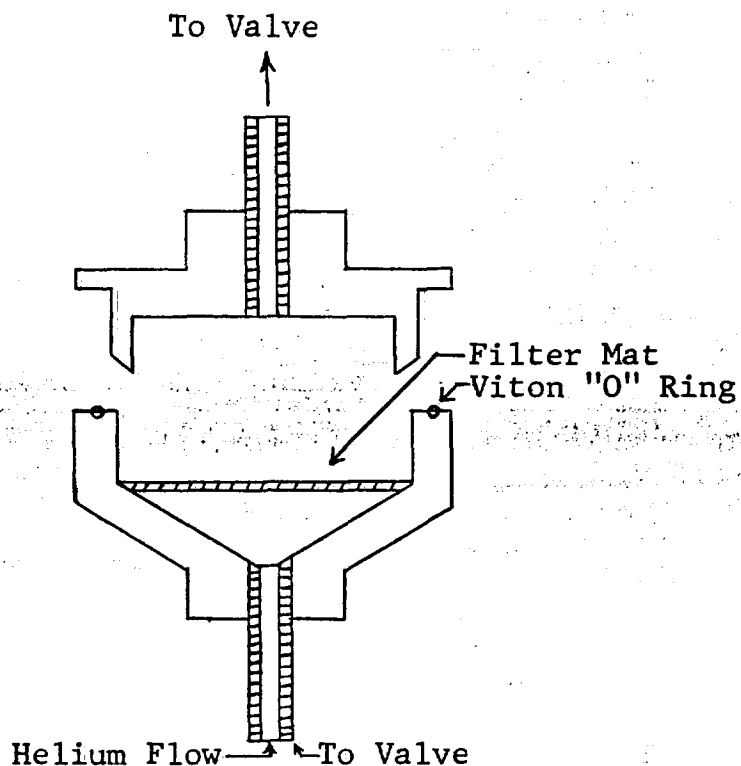
REVIEW BY R. D. HELLAMS

The gas chromatographic method presented by Mr. Lyerly for the determination of menthol, propylene glycol, nicotine, and triacetin in cigarette smoke utilizes a Cambridge filter for the collection and introduction of the smoke onto the gas chromatographic column.

The particulate phase from a single cigarette is collected on a Cambridge filter using the standard puff of 35 cc. for 2 seconds each minute. A modified Cambridge filter holder (Figure 1) used to hold the Cambridge filter is then connected to a heated sampling valve connected to the injector of the Barber-Coleman 5000 Selecta System gas chromatographic instrument which utilizes a thermal conductivity cell detector. The opposite end of the special Cambridge filter holder is connected to a valve which controls helium flow through the Cambridge filter holder.

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FIGURE 1



A fluidized sand bath at 200°C is used to heat the filter holder while the menthol, propylene glycol, nicotine, and triacetin are swept onto and collected in the initial part of the 6' x  $\frac{1}{4}$ " column (14% DEGS on Chromosorb W) at 50°C. After a ten minute sweep period the sampling valve is changed to reroute the flow.

The column temperature is programmed from 50°C to 175°C at 12°/minute for ten minutes, after which the column remains at 175°C until the final peak, triacetin, is eluted and the analysis is completed in 22 minutes.

A calibration curve was prepared by adding the four components in chloroform to a Cambridge filter with a microliter syringe and analyzing the material on this filter by the same procedure as a smoke sample.

The analysis of menthol in cigarette smoke for four brands is given in Table 1, while a typical puff-by-puff analysis of menthol in cigarette smoke is given in Table 2.

TABLE 1

Analysis of Menthol in Cigarette Smoke

<u>Brand</u>	<u>Mg. Menthol/Cigarette</u>
A	0.39
	0.43
	0.40
B	0.48
	0.49
	0.50
C	0.30
	0.29
	0.31
D	0.23
	0.21
	0.22

TABLE 2

A Typical Puff-by-Puff Analysis of Menthol in Cigarette Smoke

<u>Puff No.</u>	<u>Mg. Menthol/Puff</u>
1	0.030
2	0.031
3	0.036
4	0.036
5	0.038
6	0.045
7	0.045
8	0.045
9	0.042

Typical results for the analysis of propylene glycol, nicotine, and triacetin in cigarette smoke by the above presented gas chromatographic method are given in Table 3.

TABLE 3

Analysis of Propylene Glycol, Nicotine, and Triacetin in Cigarette Smoke by Vapor Chromatography

<u>Sample No.</u>	<u>Propylene Glycol mg./cigt.</u>	<u>Nicotine mg./cigt.</u>	<u>Triacetin mg./cigt.</u>
A	0.44	1.23	0.31
	0.42	1.21	0.32
	0.39	1.38	0.32
	0.38	1.21	0.32
B	1.01	1.45	0.25
	1.03	1.50	0.25
	0.98	1.45	0.31
	0.95	1.41	0.33

Table 4 gives a comparison of this gas chromatographic method with other methods for the analysis of propylene glycol and nicotine in smoke.

TABLE 4

Comparison of Vapor Chromatographic Analysis of Propylene Glycol and Nicotine in Smoke with Other Methods

<u>Brand</u>	<u>Propylene Glycol, mg./cigt.</u>		<u>Nicotine, mg./cigt.</u>	
	<u>VPC</u>	<u>Colorimetric</u>	<u>VPC</u>	<u>Spectrophotometric</u>
A	0.38	0.28	1.16	1.05
B	0.95	0.62	1.06	1.11
C	0.96	0.85	1.31	1.31
D	0.34	0.23	0.75	0.76

The low results for the propylene glycol by the colorimetric method were explained by the speaker as being due to the fact that in the colorimetric method the propylene glycol is not separated from the other smoke components as it is in the gas chromatographic method.

The transfer of propylene glycol and nicotine from the Cambridge filter to the gas chromatographic column versus the direct injection of these standards onto the gas chromatographic column was checked. The same peak heights occurred in the two cases.

The speaker summarized his presentation by saying that in this gas chromatographic method no chemical separation is required, quantitative data for four compounds may be made on a single cigarette, total sample from a single cigarette is used instead of aliquots, and a puff-by-puff analysis is possible for any component or for all four components.

Questions asked by the audience after the speaker's presentation of his paper are as follows:

1. Is the Cambridge filter treated? Answer: No.
2. Why did water not show on the chromatogram?  
Answer: The water was removed from the column during the 10 minute sweeping period before programming the column temperature.
3. Were these experiments done to check the quantitative collection of these four compounds on the Cambridge pad during smoking? Answer: No.
4. Was there any study done with the gas chromatographic method for the analysis of smoke where any of the four compounds was not present? Answer: No.

LICORICE FLAVORING - RAPID  
ANALYSES OF SUGARS AND GLY-  
CYRRHIZIN USING MODERN INSTRU-  
MENTAL METHODS. R. J. Morris,  
Jr., and C. Romano, MacAndrews  
and Forbes Company, Camden,  
New Jersey.

ABSTRACT

In this country, about 90 percent of the licorice flavoring is used by the tobacco industry as an ingredient in tobacco casing liquor. The most recent scheme of analysis for licorice was proposed by Dr. Percy Houseman in 1922. Recent efforts to up-date these methods have resulted in a rapid gas chromatographic analysis for sugars based on the silyl ether derivative technique developed by Sweeley and coworkers, and an infrared spectrophotometric analysis for glycyrrhizin using aqueous solutions. These analyses require about 45 minutes each and the Houseman methods require  $2\frac{1}{2}$  hours for sugars and 3 days for glycyrrhizin. The calibration of the instrumental methods is presently based on the Houseman assay so as to do as little violence as possible to the 43 years of accumulated data. The precision of the G. C. method for sugars is: reducing sugars  $\pm 0.3\%$  and cane sugar  $\pm 0.3\%$ , while the Houseman assay gives a precision of  $\pm 0.3\%$  for reducing and  $\pm 0.5\%$  for cane sugars, respectively. The precision of the IR method for glycyrrhizin is  $\pm 0.7\%$ , while the Houseman assay gives  $\pm 0.3\%$ . It is felt that the savings in time per analysis compensate for the apparent loss of the precision of the glycyrrhizin method. Present work is directed toward the determination of individual sugars and the determination of glycyrrhizinate content using pure standards.

A copy of this paper is available in the library.

1003109934

THE PHYSICAL STRUCTURE OF RECONSTITUTED TOBACCO SHEET. Raymond J. Moshy and Robert E. Lang, American Machine & Foundry Company, Springdale, Connecticut.

ABSTRACT

The development, utilization and general acceptance of reconstituted tobacco sheet in cigarette manufacture in the United States is a major technological feat, which has been accomplished within the past ten years. The impetus behind this unusually fast development, and the commercial application of reconstituted tobacco sheets, has been the savings derived from the more complete use of tobacco in cigarette manufacturing operations, in which sheet tobacco is incorporated. Details of economics, processing and use in American cigarettes are given in the paper. The virtually untouched area of the physical structure of reconstituted cigarette tobacco sheets will be discussed in the context of structure and process type; and, also in the context of structure and smoke. The physical structures of commercial reconstituted cigarette tobacco sheets will be compared and the major differences demonstrated photomicrographically. The need for including reconstituted tobacco sheet as a component of the American cigarette blend in work on cigarette smoke; and the importance of reconstituted sheet technology as a new tool of tobacco science, which can effect structural variations in tobacco sheet, unattainable with natural tobacco, are discussed in this paper.

REVIEW BY A. B. CLARKE

This paper covered the use of reconstituted tobacco in cigarette manufacture. It was reported that 63% of the purchased leaf is directly usable as cigarette filler. Figure 1 illustrates this, which is based on tobacco auction weight utilization. During 1964 cigarettes manufactured in the U.S. contained an average of 15% reconstituted tobacco. Based on this percentage, savings amounted to about 27cents/1000 cigarettes. This saving is illustrated in Figure 2.

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FIGURE 1  
CIGARETTE TOBACCO  
AUCTION WEIGHT UTILIZATION

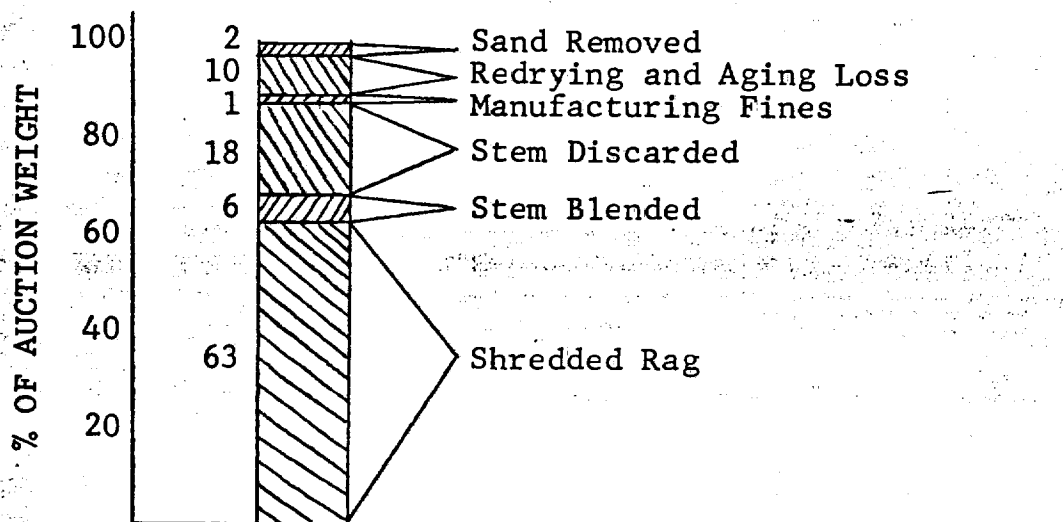
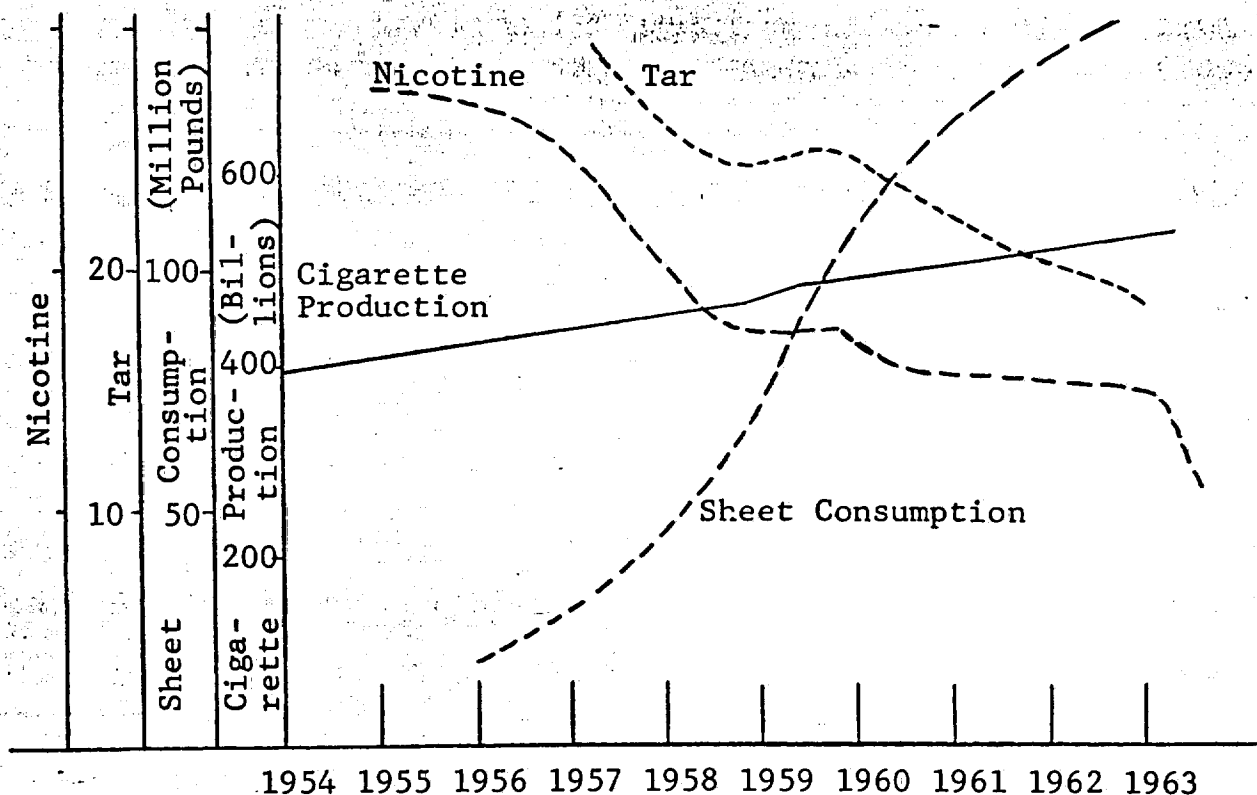


FIGURE 2  
TYPICAL SAVINGS USING MANUFACTURED  
CIGARETTE SHEET IN CIGARETTES

Cost of natural cigarette tobacco at the maker	\$1.00/lb. 2.20/1000
Cost to convert scrap (no value) tobacco to cigarette sheet	.15 - .20/lb.
Savings per pound of manufactured cigarette sheet used	.80 - .85/lb.
Savings per 1000 cigarettes using 15% manufactured cigarette sheet in cigarettes	.264 - .281/1000
<hr/>	
Cost of 20 million pound per year capacity plant	\$6 - 8 Million
ANNUAL SAVINGS	\$16 - 17 Million

There are presently five processes for preparing sheet tobacco. They are: dust-binder-dust, slurry, paper, impregnated, and extrusion. Reconstituted tobacco varies considerably in physical and chemical properties because of process differences. From a health standpoint, reconstituted tobacco has not generally been included in studies to date.

Since 1956 the use of sheet tobacco in cigarettes has increased rapidly. TPM and nicotine have been steadily decreasing. Dr. Moshy stated that this general reduction has been largely due to the addition of reconstituted tobacco. This information is illustrated in Figure 3.



See Tobacco, 162 (1), 22-38 (1966).

THE USE OF A FURNACE TECHNIQUE  
FOR STUDYING THE PYROLYSIS OF  
TOBACCO

C. I. Ayres and R. E. Thornton.  
Research & Development Establish-  
ment, British-American Tobacco  
Company, Ltd., Southampton, U.K.

ABSTRACT

A technique has been developed in which small samples of tobacco are pyrolysed in a furnace, and the yields of smoke components thus obtained are correlated with the deliveries obtained when the tobacco is burnt as a cigarette. Such a technique should include very rapid heating of the sample, and efficient collection of the pyrolysis products with avoidance of secondary pyrolysis. Following studies of various designs, these requirements have been met by the use of a short length mullite tube furnace. With this equipment, the production of phenols from tobacco has been studied. By examining the effects of sample size, pyrolysis time, pyrolysis temperature and carrier gas flow on the yield of phenols, standardized conditions have been determined at which a direct correlation can be made between the yields of phenols produced by pyrolysing a tobacco, and the product efficiency (E phenols) of the same tobacco, calculated from cigarette smoking data. Thus, it appears that the delivery of phenols from a tobacco in cigarette form can be predicted by the furnace technique. Initial studies are also reported on the possible precursors of phenols in cigarette smoke.

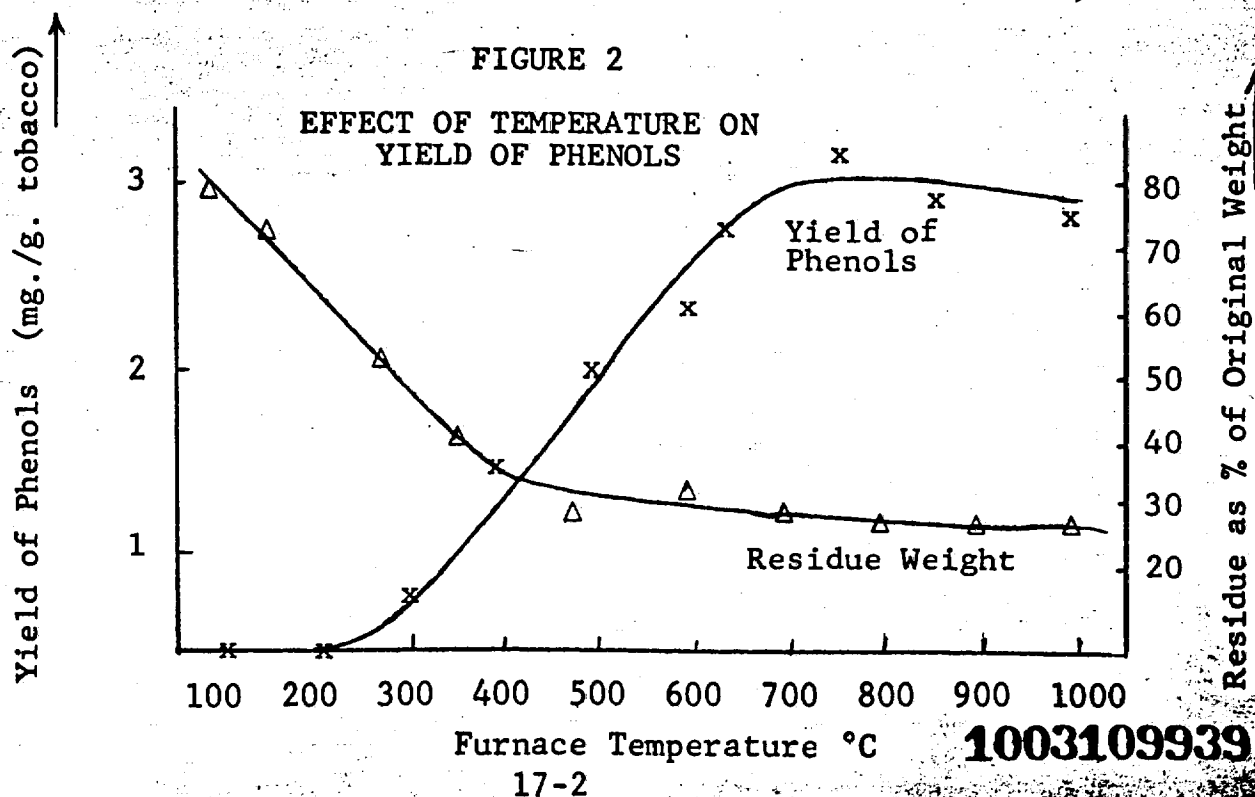
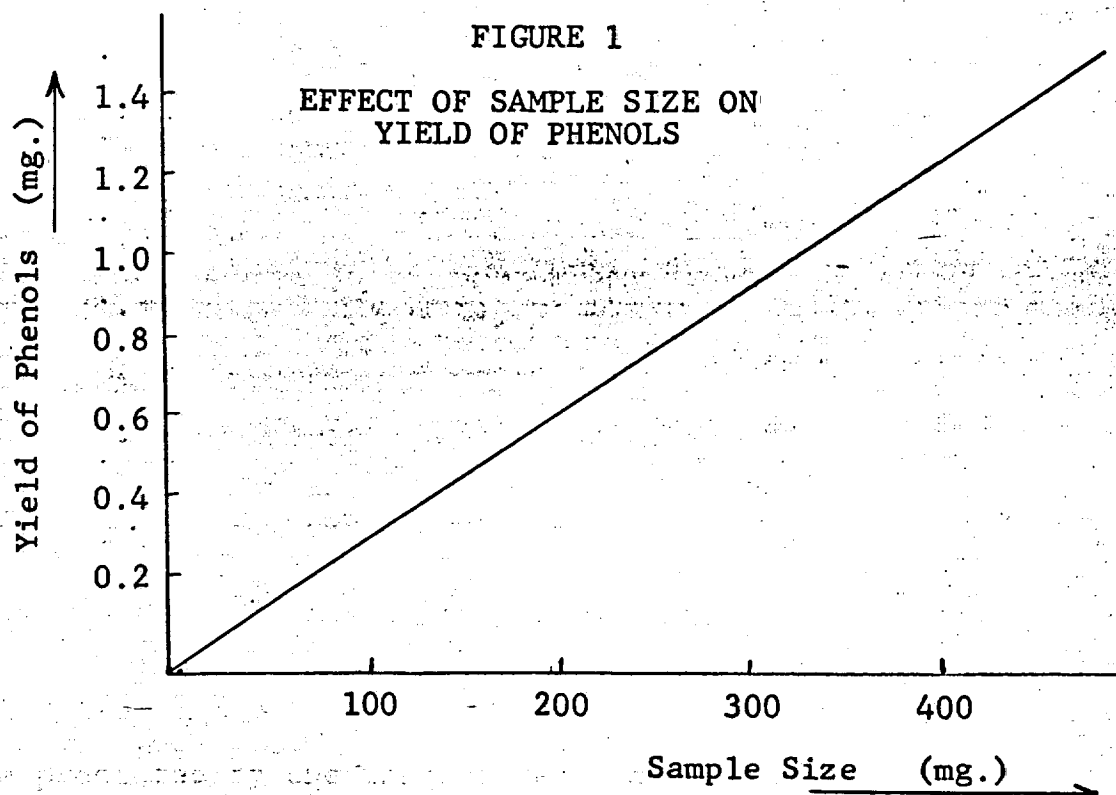
REVIEW BY M. L. GILFOYLE

A method was developed in which a direct correlation could be made between the yields of phenols produced by pyrolyzing tobacco and the product efficiency (E phenols) of the same tobacco calculated from cigarette smoking data.

A short length mullite tube furnace was used for pyrolysis. 150  $\pm$  25 mg of tobacco was placed in a porcelain boat and positioned in the furnace with a loading rod. The N<sub>2</sub> flow was regulated at 600 ml/min. The tobacco was heated rapidly to 800°C and the sample was pyrolyzed for 60 sec., although pyrolysis was complete in 20 sec. The pyrolyzate was swept off rapidly with the carrier gas to prevent secondary pyrolysis. The product was collected in a spiral trap at dry ice temperature. The trap was backed by a Cambridge filter.

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There was a direct relationship between the size of the tobacco sample and the yield of the phenols. A large sample gave a large yield of phenols.

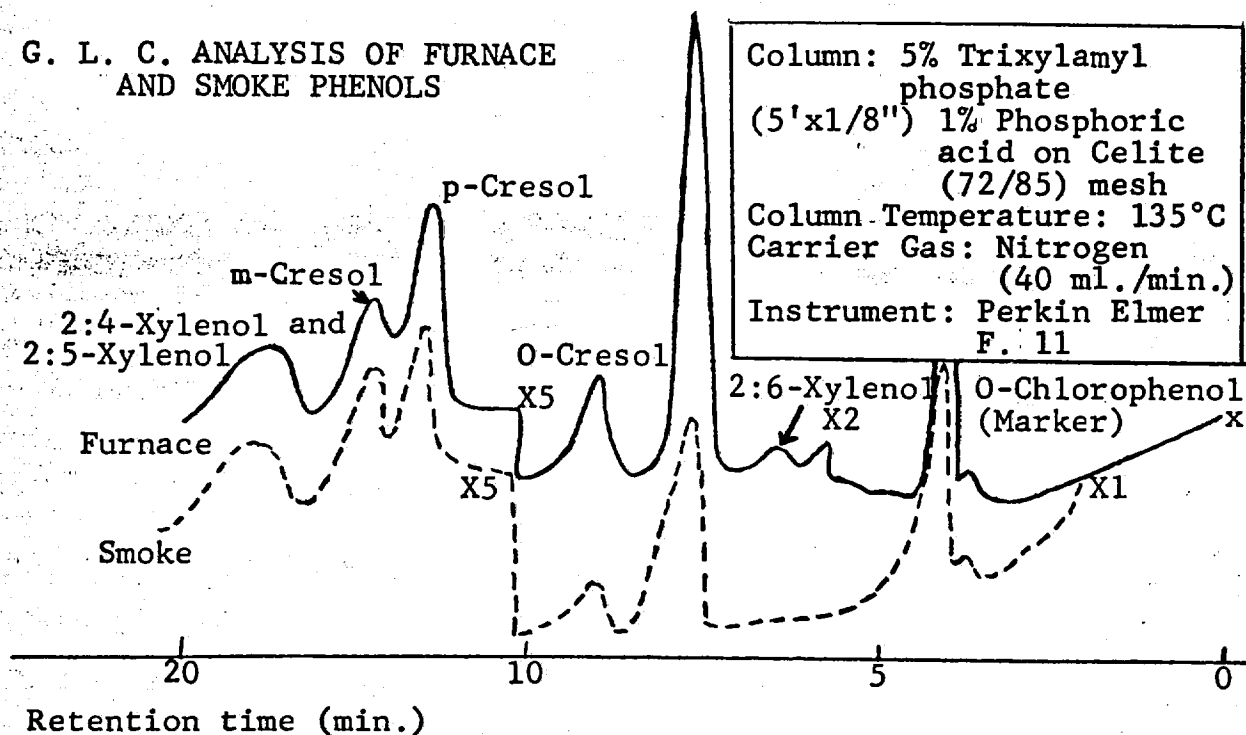


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As shown in Figure 2, the yield of phenols was at a maximum at 800°C. The residue weight was at a constant value between 500° and 1000°C.

FIGURE 3

G. L. C. ANALYSIS OF FURNACE  
AND SMOKE PHENOLS

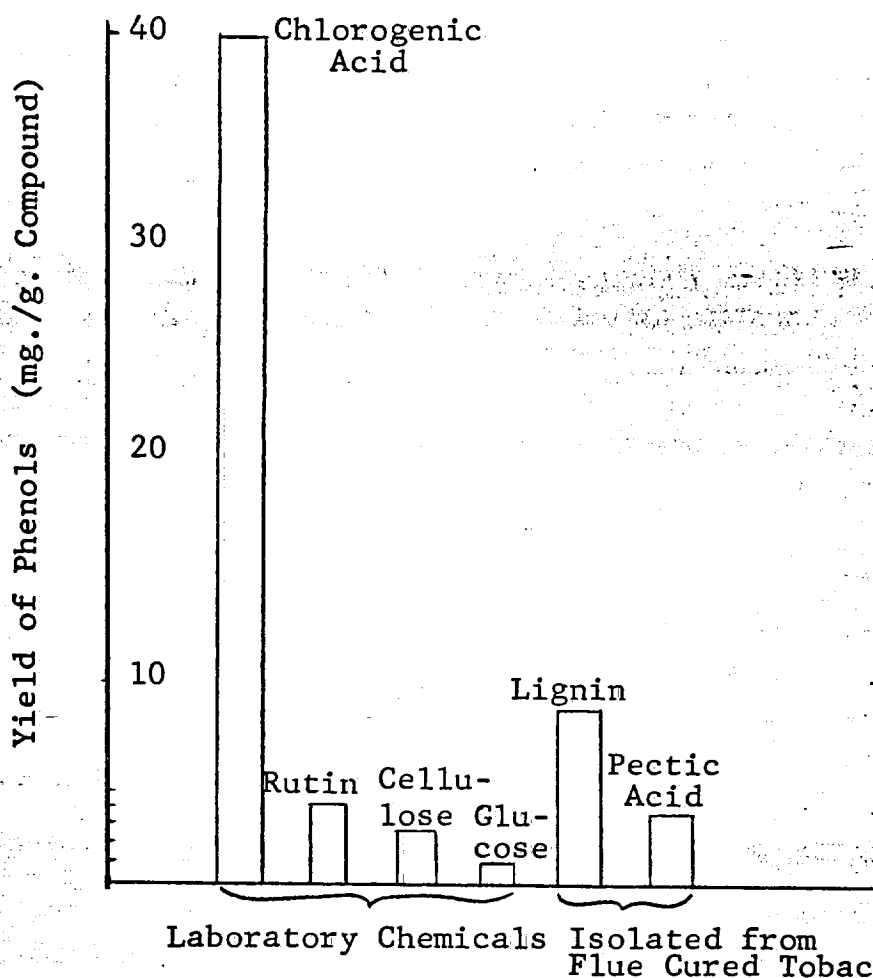


The large unmarked peak is phenol.

By this method the yield of phenols from tobacco when burned in cigarette form can be predicted by the furnace technique.

FIGURE 4

## PRECURSORS OF PHENOLS



Chlorogenic acid appears to be the largest precursor of phenols in tobacco. Flue-cured tobacco contains free chlorogenic acid. When tobacco is pyrolyzed, chlorogenic acid converts to phenol at a much greater rate than do other components.

Addition of Chlorogenic Acid to the Cigarette

<u>Tobacco</u>	<u>Additive</u>	<u>Deliveries of Phenol μg/cigt.</u>
Burley	0% (control)	215
Burley	3% (chlorogenic acid)	255

The phenol deliveries were determined gas chromatographically.

THE PYROLYSIS OF POLYPHENOLIC  
PIGMENTS OF TOBACCO.

O. T. Chortyk, W. S. Schlotz-  
hauer, R. L. Stedman, USDA,  
Eastern Utilization & Research  
Development Division, Phila-  
delphia, Pennsylvania.

ABSTRACT

A pyrolytic study was made on certain high molecular weight polyphenolic tobacco pigments, which were isolated from Turkish tobacco and are known to contain rutin, chlorogenic acid, and amino acids. Pyrolysis of the dry pigment was conducted at 850°C., the approximate burn temperature of a cigarette. Most of the pyrolytic products were gases and volatiles not condensed in Dry Ice traps. The condensed products were fractionated into various groups, including a polynuclear aromatic hydrocarbon fraction. Using the usual isolation and identification procedures (thin-layer and paper chromatography, ultraviolet and fluorescence spectra), more than 12 polynuclears were found including anthracene, phenanthrene, fluoranthene, pyrene, chrysene, and benzo(a)pyrene as the major constituents. The yields of polynuclears obtained from the pigment were relatively high, e.g. about 1 mg. of benzo(a)pyrene from 1 g. of pigment. The use of a new gas solid chromatographic system for the separation of the higher polynuclear hydrocarbons in this study will be discussed.

REVIEW BY M. D. EDMONDS

During a conversation with Dr. Stedman concerning this paper, he stated that their pyrolysis work on polyphenolic pigments was in its preliminary stages.

The theory behind their pyrolysis is based on the formation of unstable free radicals which in turn undergo further reactions to form polynuclear hydrocarbons.



Polyphenolic pigments were isolated from Turkish tobacco. The pigment fraction, consisting of rutin, chlorogenic acid, and certain amino acids, accounted for 4% by weight of the total sample.

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The dried pigment was pyrolyzed at 850°C. The condensable products were collected and separated into acidic, basic, and neutral fractions. No work was reported for the acidic and basic fractions.

The neutral fraction was extracted with cyclohexane and separated into oxygenated neutrals, hydrocarbons, and other volatiles. The hydrocarbon fraction was separated into a fraction containing compounds of carbon and hydrogen and a fraction containing compounds of carbon, hydrogen and nitrogen. The latter fraction was not discussed.

The hydrocarbon fraction containing carbon and hydrogen was taken up in nitromethane and chromatographed on silica gel. The numerous fractions which were collected were chromatographed on paper and on thin layer plates (no mention of kind) and developed in methanol-ether-water or in toluene-ethanol-water systems. The following polynuclears were identified by  $R_f$  values and ultraviolet and fluorescence spectra:

benzene	pyrene*
naphthalene	1-methylpyrene
anthracene*	chrysene*
phenanthrene*	benzo(a)pyrene*
fluoranthene*	* Major constituents

The quantitative data showed that the pigment produced BaP at a concentration of 1000 times that found in cigarette smoke.

A new solid gas chromatographic system for resolving higher molecular weight polynuclears was described. This was designated as "lithium chloride substrate," consisting of 20% lithium chloride on Chromosorb P.

A mixture of 28 polynuclears was resolved on the  $\text{LiCl}_2$  substrate. The operating conditions were not given.

The advantages of this new substrate are: rapid resolution, quantitative data, decreased losses, and large-size sample analysis possible.

THE CONTRIBUTION OF TOBACCO CONSTITUENTS TO PHENOL YIELD OF CIGARETTES.

A. W. Spears, J. H. Bell and A. O. Saunders, Research Division, P. Lorillard Company, Greensboro, North Carolina.

ABSTRACT

Phenol has been a reported component of cigarette smoke for many years, but no occurrence in American tobaccos has been reported. Obviously, phenol is formed during the smoking process and differences in phenol yield may be found between tobacco types (Bright and Burley). Pyrolysis studies were carried out at various temperatures in both air and nitrogen with Bright and Burley tobaccos until conditions were found which gave the same ratio of phenol yield as obtained with the respective cigarettes on smoking. Using these conditions, leaf extracts and leaf components were pyrolyzed and phenol yields determined. Both the method and data will be presented. On the basis of the pyrolysis studies, uniformly labeled  $C^{14}$  glucose was added to tobacco and after preparation of cigarettes and smoking, the incorporation of radioactivity into phenol was determined. Data will be presented. Both the data from pyrolysis studies and the addition of  $C^{14}$  glucose to cigarettes will be discussed, and the role of carbohydrates as precursors of phenol will be evaluated. The data provide some information with respect to the mechanism of phenol formation and some inferences will be made. In general, it is thought that these techniques provide a means for elucidating smoke precursors, but certain limitations exist which will be indicated in the discussion.

REVIEW BY M. D. EDMONDS

A method was presented for the pyrolysis of individual tobacco constituents and burley and bright tobaccos, and a measurement of phenol was obtained from the pyrolysates.

The pyrolysis apparatus was quite similar to the one used here at the Research Center. The samples were pyrolyzed in both oxygen and nitrogen atmospheres. The trapped samples were collected at dry ice-acetone temperature. The phenol content was measured by the procedure of A. W. Spears, Quantitative Determination of Phenol, Anal. Chem., 35, 320-322 (1963).

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The following data were presented:

Percent Conversion To Phenol

	<u>Air Atm-100 ml/min.</u>		<u>N<sub>2</sub>-100 ml/min.</u>
Glucose	$5.0 \times 10^{-2}$	$7.7 \times 10^{-2}$	$9.0 \times 10^{-2}$
Sucrose	$6.7 \times 10^{-2}$	$6.2 \times 10^{-2}$	$8.2 \times 10^{-2}$
Starch	$2.2 \times 10^{-3}$	$3.0 \times 10^{-2}$	$10.0 \times 10^{-2}$
Cellulose	$2.4 \times 10^{-2}$	$8.0 \times 10^{-3}$	$4.0 \times 10^{-2}$
Pectin	$2.6 \times 10^{-2}$	$1.7 \times 10^{-2}$	$3.9 \times 10^{-2}$
Rutin	$8.0 \times 10^{-3}$	$4.0 \times 10^{-3}$	$2.8 \times 10^{-2}$
Chlorogenic Acid	---	$8.0 \times 10^{-3}$	---
Flue Cured Tobacco	$5.0 \times 10^{-2}$	$2.9 \times 10^{-2}$	$20.0 \times 10^{-2}$
Burley Tobacco	$4.7 \times 10^{-2}$	$2.6 \times 10^{-2}$	$14.0 \times 10^{-2}$
Stationary Furnace 1 =	315°C	777°C	315°C
Stationary Furnace 2 =	320°C	562°C	320°C
Mobile Furnace =	685°C	685°C	685°C

There was no significant difference in the amount of phenol from flue-cured and burley tobaccos when pyrolyzed in air at 315°C or at 777°C. In a nitrogen atmosphere at 315°C the difference in phenol between flue-cured and burley was in the same ratio as the difference in the smokes of the respective tobaccos (1.6 to 1).

A sample of flue-cured tobacco was extracted with hexane and then with 75% ethanol. The ethanol extract was chromatographed on a Celite column. The column was washed with methanol, acetone, ether, and hexane, respectively. I did not get the final figures on the amounts of phenol, but the greater the polarity of the solvent, the greater the yield of phenol. It was stated that the phenol yield was due partially to carbohydrates.

A four gram sample of flue-cured tobacco was extracted with 75% ethanol. To the ethanol extract  $5.0 \times 10^{-5}$  curie of C<sup>14</sup>-labeled glucose was added. The ethanol was evaporated and the sample was lyophilized. Four cigarettes were prepared and smoked. By radio assay this gave  $1.93 \times 10^{-7}$  counts/min. for phenol for the four cigarettes. It was stated that the uniformly labeled C<sup>14</sup> glucose contributed 41% to the total yield of phenol in sidestream and mainstream smoke.

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It was mentioned that the results for phenol may be higher than normal due to air oxidation, pyrolysis of lignins, pyrolysis of oxygenated compounds, and to carbon exchange. Also, flue-cured tobacco may have a catalytic effect on the formation of phenol.

THE EFFECT OF pH ON CIGARETTE  
SMOKE FILTRATION.

W. B. Fordyce and H. G. Horse-  
well, Research & Development  
Establishment, British-American  
Tobacco Co., Ltd., Southampton,  
U.K.

ABSTRACT

In a previous paper (C. I. Ayres and S. R. Evelyn - 18th Tobacco Chemists' Conference, 1964) it was shown that the selective filtration of nicotine relative to total particulate matter (T.P.M.), by conventional cellulose acetate filters, is dependent upon the pH of the smoke presented to the filter. This phenomenon has now been studied in greater detail using cigarettes, the tobacco compositions of which have been varied in order to give smoke of differing pH values, and a range of filters treated so as to cover a range of pH. The results from this study have been interpreted in terms of a vapour concentration gradient between the smoke droplet and the filter surface, the slope of which is predominantly determined by the pH of the smoke and filter. It has been concluded, therefore, that the differing degrees of selective filtration of nicotine and certain other smoke constituents arise from (i) changes in the distribution of these smoke constituents between the vapour and particulate phases, (ii) the influence of pH in determining this distribution, and (iii) the rapid equilibration of the vapour-particulate distribution following removal of a smoke constituent from the vapour phase by filtration.

REVIEW BY J. S. OSMALOV

NOTE: This paper was read, and because of the large amount of material covered it was difficult to take very complete notes. The report on this talk, outlined below, covers only a portion of the total talk. A copy of this paper was requested from the authors; if permitted, they will send us one. They indicated the paper would be published.

In a previous paper Ayres and Evelyn showed that the selective filtration of nicotine was dependent on smoke pH. Selective filtration was studied in greater detail in this work, with emphasis on smoke pH, total nicotine, total alkaloid fraction, and steam volatile acids. For the experimental work,

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changes in smoke pH were accomplished by use of burley tobacco (high pH), flue-cured tobacco (low pH), and blends of these tobaccos. To achieve the desired filter pH, a non-volatile acid or base was used on the filter, the filter having been neutral before treatment. Cigarettes and filters used in all experiments were selected for constant weight and pressure drop. Particulate efficiency was determined from measurement of the oven-dried smoke condensate.

#### CA Filters

Particulate efficiency was found to increase as the pH of the filter increased. The best efficiency was achievable with the more acid smoke and an alkaline filter.

The filtration of nicotine was accounted for by three mechanisms as follows:

1. Mechanical interception.
2. Sorption from the vapor phase.
3. Distillation from particles to filter (and distillation from the filter to particles in certain instances).

Concentration of free nicotine in the vapor is proportional to the nicotine in the smoke. In both flue-cured and burley the concentration is small.

With neutral filters the mechanism for nicotine filtration is distillation; the efficiency is less than that with acid filters since the driving force is decreased with the neutral filter. With alkaline filters nicotine salts trapped on the filter go to free nicotine. This free nicotine opposes distillation of nicotine from the smoke particles to the filter and instead will distill from the filter to the particles.

The driving force or filtration potential of nicotine by acetate was illustrated in the following manner:

FIGURE 1

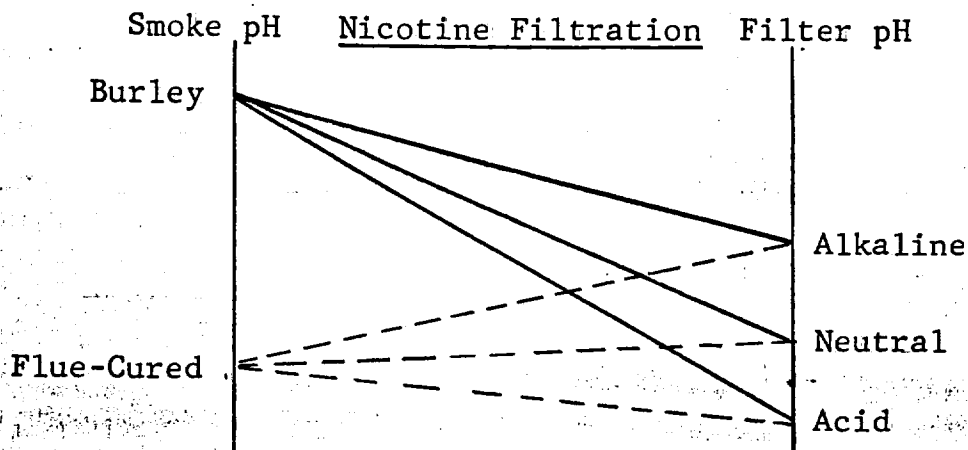


Table I summarizes nicotine filtration results obtained with various pH tobaccos and filters.

TABLE I

Cigarette Composition	Cigt. #1	Cigt. #2	Cigt. #3	Cigt. #4	Cigt. #5
% Burley	100	75	50	25	0
% Bright	0	25	50	75	100
pH of TPM	7.3	6.7	5.9	5.4	5.3
% Free Nicotine	23	6	1	0.3	0.2
<u>% Nicotine Retained</u>					
<u>Filter Type</u>					
Acid Acetate	26	18	11	7	3
Neutral Acetate	7	4	2	1	0
Alkaline Acetate	5	0	-1	-3	-4
Paper	3	1	1	0	0

#### Steam Volatile Acids

Filtration of the steam volatile acids increased with increasing pH of the filter. The authors reported that acetic acid accounts for 50% of these steam volatile acids. The alkaline filter holds the acids as salts. The filtration potential for these acids by acetate filters was illustrated as follows in Figure 2 and Table 2.

FIGURE 2

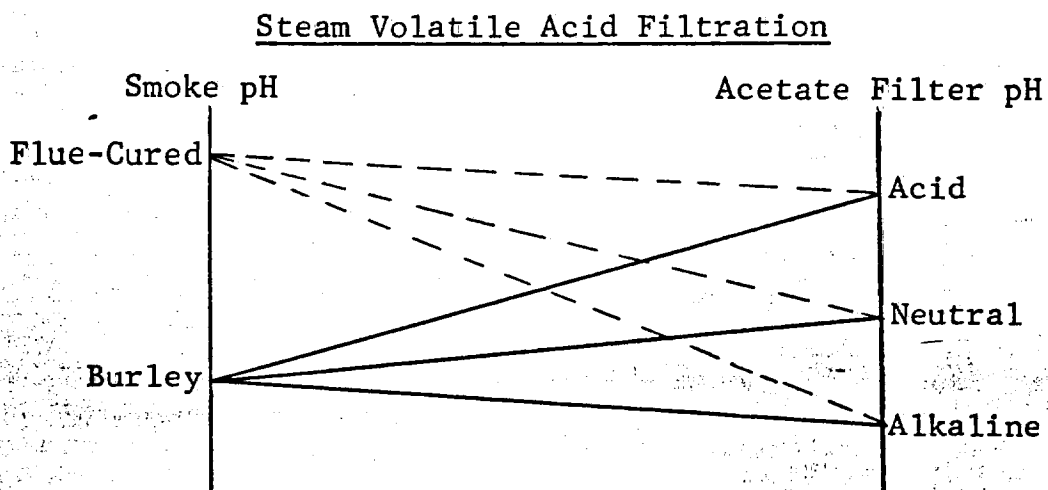


TABLE II

Cigarette Composition	Cigt. #1	Cigt. #2	Cigt. #3	Cigt. #4	Cigt. #5
% Burley	100	75	50	25	0
% Bright	0	25	50	75	100
pH of TPM	7.3	6.7	5.9	5.4	5.3
% Free Acids	0.3	1	6.5	18	26
<u>% Free Acids Retained</u>					
<u>Filter Type</u>					
Acid Acetate	-9	-7	-1	-1	3
Neutral Acetate	-2	-1	4	11	15
Alkaline Acetate	6	22	29	29	29
Paper	-17	-8	-3	4	4

Other comments included the following: As smoke pH decreases, the efficiency of CA goes up for phenols; however, filter surface area is more important than pH in phenol removal from smoke. For wet tar, as smoke pH decreases filtration decreases.

The pH of smoke was determined by washing the Cambridge pad with water and measuring the pH of the solution.

There was selective filtration of nicotine relative to TPM on various types of filters. The different degrees of selective filtration of nicotine and certain other smoke constituents arise from (1) changes in the distribution of these smoke constituents between the vapour and particulate phases (2) the influence of pH in determining this distribution, and (3) the rapid equilibration of the vapour-particulate distribution following removal of a smoke constituent from the vapour phase by filtration.

Paper No. 21

FACTORS AFFECTING THE DELIVERY  
OF CIGARETTE SMOKE VAPOUR PHASE.  
M. L. Reynolds, The Imperial  
Tobacco Company, Limited,  
Bristol, England.

ABSTRACT

The deliveries of carbon monoxide, methane, hydrogen, hydrogen cyanide, aldehydes, and "total organic vapour phase" (defined in terms of a Cambridge filter and a flame-ionization detector) have been measured for cigarettes of various lengths. The contribution, and relative importance, of filtration, repyrolysis, ventilation and diffusion to the variations in delivery with cigarette length of these components have been assessed.

REVIEW BY R. M. WILEY

Diffusion loss is a dominating factor in controlling vapour phase delivery from a normal plain cigarette. Delivery of a component at the mouthpiece of a cigarette shows an exponential dependence on the length of cigarette remaining. The exponent is inversely proportional to the smoking rate. Thus, changes in chemical composition of smoke at the mouthpiece of a cigarette produced by different smoking regimes can arise in part through the physical process of diffusion loss.

A copy of this paper is available in the library.

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Paper No. 22

THE SURFACE AREA OF FIBROUS  
FILTERS.

C. H. Keith and C. F. Delaet.  
Celanese Fibers Marketing  
Company, Charlotte, North  
Carolina.

ABSTRACT

Among the parameters controlling the filtration properties and draw resistance of cigarette filters, the surface area of the fibrous medium is quite important. In the past, it has been difficult to accurately measure this quantity because of the relatively small surface involved. With the extension of the Brunauer, Emmett and Teller gas adsorption technique to low specific surface area materials by the use of Krypton gas, such measurements have become practical. Using this technique, a variety of cellulose acetate filters have been examined, and the fiber surface area correlated with other filtration parameters. The effect of fiber size, as indicated by denier per filament, fiber cross section, and bonding agents on the specific surface of the acetate filtering media are presented and discussed. For comparative purposes, data on the surface area of tobacco and paper are also presented.

A copy of this paper is available in the library.

1003109953

Paper No. 23

DETERMINING SELECTIVE EFFICIENCY  
IN CIGARETTE CHARCOAL.

M. F. Kranc, D. D. Tiggelbeck,  
and John Lutchko, Pittsburgh  
Activated Carbon Company, Pitts-  
burgh, Pennsylvania.

ABSTRACT

Quantitative measurements have been performed with activated carbon filters to determine adsorptive efficiency and breakthrough characteristics for ciliastatic gases. This technique is based upon a gas chromatograph using a flame ionization detector. The instrument has been modified to allow use of a synthetic filter in place of the separation column. The gases studied were acetaldehyde, acrolein and hydrogen cyanide. Nitrogen was used as the second component. Variables included pore size distribution, activity level, apparent density, raw material and impregnants. The data presented include equilibrium isotherms as well as breakthrough curves, showing the effects of the variables. The direction of further work as indicated by these tests will be discussed.

A copy of this paper is available in the library.

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Paper No. 24

ASPECTS OF THE FLUID DYNAMICS OF  
CIGARET SMOKE FILTRATION.

Waller George, Celanese Research  
Laboratories, Summit, New Jersey

ABSTRACT

The first part of this discussion reviews the physics of critical Reynolds numbers. The Navier-Stokes equations for fluid dynamics are used to introduce a discussion of the statistical character of turbulent fluid flow. The concept of turbulence of different "scales" is introduced. The critical Reynolds number for flows around obstacles in open, porous beds such as cigaret filler is discussed in terms of data available in the literature. It is suggested that this number lies between 10 and 200, with the probability of a value substantially smaller. Simple calculations are shown which indicate that the flow during a typical cigaret puff lies in the indicated range and may contain, therefore, a substantial unstable turbulent component. This component is suggested as being contained in the free stream entering the cigaret filter. The consequences of the free stream turbulence in the filter are discussed with specific reference to the movement of smoke aerosols towards filter components. It is shown that "micro" turbulence produces a "macro-Brownian" type of motion which is formally analogous to that proposed by Langmuir as governing the filtration of toxic smokes by gas masks. The state of the fluid flow envisioned by Langmuir is contrasted with that presently envisioned as occurring in the cigarette filter.

A copy of this paper is available in the library.

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Paper No. 25

AN IMPROVED, AUTOMATIC SMOKING  
MACHINE FOR THE LARGE SCALE  
COLLECTION OF CIGARETTE SMOKE  
CONDENSATE.

Robert E. Lang and Raymond J.  
Moshy, American Machine &  
Foundry Company, Springdale,  
Connecticut.

ABSTRACT

An improved, fully automatic smoking machine has been designed and built for the large scale collection of cigarette smoke condensate. In addition to its excellent performance, outstanding characteristics are simplicity of design, versatility, and truly-automatic operation. This machine smokes regular (70 mm) cigarettes at the rate of 540 per hour, subjecting them to the normal puffing sequence of two seconds duration, 35 ml. volume, once a minute. A maximum of ten puffs can be taken on each cigarette. Conditions of smoking, such as puff duration, puff volume, puff frequency, and number of puffs can be changed with only minor adjustments. In addition, the machine can be operated with either "free" or "restricted" smoking. Cigarettes are automatically loaded, lighted with a proximity-type lighter, puffed, using a modified constant time principle and, after the desired number of puffs, automatically ejected and extinguished. The operator's function has been reduced to one of merely keeping the hopper filled and being present in the event of some minor malfunction. Smoke condensate comes in contact only with glass, Teflon and stainless steel. The design is such that the condensate manifold can easily be disassembled and cleaned. The details of construction and operation of the device will be discussed. Puff profile and smoke condensate yields will be compared with those from other types of smoking machines.

A copy of this paper is available in the library.

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SMOKING MACHINES FOR THE ANALYSIS  
OF THE VAPOR PHASE OF TOBACCO  
SMOKE.

J. R. Newsome, V. Norman and C. H.  
Keith, Research Department, Lig-  
gett and Myers Tobacco Company,  
Durham, North Carolina.

ABSTRACT

Three multiport smoking machines, designed for the direct determination of various smoke vapor phase components without the utilization of condensation traps, are described. The machines are solenoid-operated and use Cambridge filters to remove most of the particulate phase, a vacuum reservoir to give the desired puff volume and flow limiting orifices to control puff flow velocity and duration. One of the modifications, a four-port machine, is designed for use with bubble traps for the determination of the total amount of gases such as hydrogen cyanide and hydrogen sulfide in smoke from a given number of puffs. The second, five-port machine is designed for puff-by-puff analysis of gases such as nitric oxide which cannot be conveniently collected with bubble traps and impingers. The third modification provides a simultaneous composite sequential puff consisting of the second through the seventh puff from six different cigarettes. Its use for gas chromatographic smoke vapor analysis is discussed. Typical data obtained with the smoking machines are presented.

REVIEW BY R. M. WILEY

Three multiport smoking machines, of four, five and six ports, were designed for the direct determination of various smoke vapour phase components without the utilization of condensation traps. The machines are solenoid operated and use Cambridge filters to remove most of the particulate phase. A vacuum reservoir gives the desired puff volume and flow limiting orifices control puff flow velocity and duration.

A four port machine is designed for use with bubble traps for the determination of the total amount of gases such as hydrogen cyanide and hydrogen sulfide in smoke from a given number of puffs. The machine puffs every fifteen seconds with a total cycle of one minute.

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A five port machine is designed for puff-by-puff analysis of gases such as nitric oxide which cannot be conveniently collected with bubble traps and impingers. Here, 200 cc are puffed into the reservoir, and a 40 cc aliquot is puffed into the sample flask. After 5 seconds, the sample flask is replaced with a new flask and the cycle is repeated. The cigarettes tested should be draw selected.

A six port machine provides a simultaneous composite sequential puff consisting of the second through the seventh puff from six different cigarettes. Its use for gas chromatographic smoke vapor analysis was discussed.

Paper No. 27

GAS CHROMATOGRAPHY OF CIGARETTE  
SMOKE, PART IV; SEPARATION ON  
GLASS CAPILLARY COLUMNS.

Kurt Grob, F. J. Burrus & Cie,  
Boncourt, Switzerland, and  
Department of Organic Chemistry,  
University of Zurich.

ABSTRACT

Glass capillaries offer the following advantages over steel capillaries: As stated in part III of this study, some of the less stable smoke components do not pass steel capillaries; they are, however, detected in the effluents of glass capillaries. Retention on the glass surface is weaker than on the steel surface. Satisfying chromatograms are therefore obtained from single columns, since less rapid temperature programming is required. The low adsorption activity of the glass surface permits good separation on liquid phases of moderate polarity. The i.d. of glass capillaries drawn in our own laboratory can easily be varied and thus adjusted to particular requirements. For the analysis of fresh smoke, 0.35 mm is best suited. Preparation and coating of the glass capillaries as well as collecting of the vapor phase, and gas chromatographic techniques are described. Typical chromatograms are presented, covering, with improved separation, the same range of the vapor phase treated in part III of this investigation. Differences between results obtained from fresh and from condensed smoke are discussed.

REVIEW BY F. E. RESNIK

Dr. Grob of the University of Zurich presented a very interesting paper using glass capillary columns for the separation of cigarette smoke. He felt that a glass capillary column had an advantage over a steel capillary in that some of the less stable smoke compounds do not pass through steel capillaries and retention of these compounds on the glass surface is weaker. The technique used by Grob to coat the glass columns with a polar liquid was first to coat the capillary with a millimicron film of carbon. The polar liquid phase was then coated at a film level of 0.01 micron. Grob used a 150 meter column of 0.03 mm diameter to obtain 480,000 theoretical plates. Analysis of smoke gave 180 peaks on the glass capillary column whereas previously he had obtained 120 peaks using the steel column.

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Dr. Grob emphasized the need to analyze cigarette smoke directly without collection. He felt that the cigarette smoke changes when it is allowed to stand, even at reduced temperatures. He suggested analyzing the 6th puff, since it is an average of the entire cigarette, by subjecting this puff to direct gas chromatographic separation. He is suggesting that the vapor phase smoke be handled as quickly as possible, on the order of 0.1 to 0.2 second.

#### REVIEW BY R. D. HELLAMS

Advantages of glass capillaries versus steel capillaries for the analysis of cigarette smoke were discussed, as indicated in the above abstract.

The liquid film of 1-2 microns thickness necessary on a glass capillary column is 20 times less thick than that necessary on steel capillaries to cover the active sites. The reduced adsorbance of glass permits the use of longer columns. The viscosity of the thinner film on the glass capillary is less important, and the use of a less polar phase on the glass capillary permits longer life of the column.

A practical rule suggested by the speaker is that if a separation is best on a steel capillary column at 150°C, then comparable work could be done at 100°C on a glass capillary column.

The disadvantage of the glass capillary is that it is a weaker adsorber than steel. A good film can be obtained with a non-polar liquid phase. The speaker noted that polar liquids form a coherent film on a glass surface which has first been covered by an approximately 0.001  $\mu$  layer of carbon black. Such pretreated glass capillaries show equal or better separation efficiencies than steel capillaries and operate at lower temperatures, due to their lower adsorption activity.

Several gas chromatograms with unlabeled peaks were shown for fresh smoke comparing the 6th and 11th puffs of a cigarette. One chromatogram appeared to be labeled as being obtained with a 100 meter capillary column of 0.32 mm ID, while the other was obtained with a 130 meter capillary column of 0.36 mm ID. These chromatograms were intended to indicate peaks seen on a glass capillary chromatogram which had not been seen - or seen only slightly - on a steel capillary chromatogram. (See part III of this investigation.)

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Dr. Grob stressed the necessity of washing all tubing between gas chromatographic analyses of gas phase cigarette smoke because smoke constituents are adsorbed heavily and will influence subsequent analyses.

A specially designed 500 ml syringe connected to a Cambridge filter holder used by the speaker in this work for the collection of gas phase cigarette smoke was constructed by a Swiss watchmaker. A notched rod connected to the plunger is marked for individual puffs which are taken by hand at one minute intervals. The smoke is then pushed through the sampling valve. This method provides the advantage of analyzing the smoke from a single cigarette representing the average composition of smoke from the whole cigarette.

The use of the syringe for collecting the vapor phase of cigarette smoke from a whole cigarette is a compromise between analyzing vapor phase cigarette smoke immediately after smoking and after condensation of the smoke. Diacetyl is a possible indicator of the alteration smoke may undergo by any handling. Condensed smoke dissolved in ether and analyzed by gas chromatography showed the diacetyl had been reduced 2 1/2 times with respect to benzene.

A question from the audience concerned the sample size used in the glass capillary work for the analysis of cigarette smoke. Dr. Grob answered that this depends on the column diameter but that he used a 2 ml sample loop with a sample split of 1:50 for the work presented at this meeting.

Paper No. 28

CILIASTATIC COMPONENTS IN THE  
GAS PHASE OF CIGARETTE SMOKE.  
Ted R. Walker, John E. Kiefer,  
Tennessee Eastman Company,  
Kingsport, Tennessee.

ABSTRACT

The ciliastatic nature of the gas phase of cigarette smoke was studied by a known procedure--stroboscopic measurement of the cilia motion of the clam gill. The method was modified in an attempt to determine which of the smoke components contributed to the ciliastasis. In addition, the effects of some experimental selective filters on the activity of cigarette smoke were studied. For determining the active components, the gas phase was separated into two fractions, condensed gases and permanent gases, distinguished by whether they condense at dry ice temperature. While both fractions inhibited the ciliary beat, the condensed portion was the more potent and was, therefore, studied in more detail. To determine the ciliastatic components of the condensed gases, a gas chromatographic technique was devised which employed dual detectors--a thermal conductivity cell and a clam gill. The condensed gases after being vaporized passed successively through a chromatographic column, the thermal conductivity cell, and across the clam specimen. When the ciliastasis curves were superimposed on the gas chromatographic curves, the acrolein region was found to be most active. The hydrogen cyanide and acetaldehyde regions also strongly inhibited the ciliary motion.

A copy of this paper is available in the library.

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Paper No. 29

BENZYL-BENZOATE IN CIGARETTE  
SMOKE.

I. Schmeltz, R. L. Stedman,  
W. J. Chamberlain, USDA,  
Eastern Utilization Laboratory,  
Philadelphia, Pennsylvania.

ABSTRACT

Silicic acid chromatography of the nitromethane-soluble portion of the neutral fraction of smoke condensate from domestic cigarettes yielded a residue which showed absorption in the infrared region of the spectrum attributable to "ester" and NH or -OH. Gas chromatography of the residue on SE-30/glass beads indicated the presence of at least 20 components, with major peaks eluting at 140° and 185°C. Infrared spectral analysis of the later peak was indicative of an aromatic ester; additional data obtained by mass spectroscopy, hydrolysis, and retention time studies showed the peak to contain benzylbenzoate (400 µg/100 cigarettes). This ester has not been reported previously in cigarette smoke. The gas chromatographic peak eluting at 140°C. was identified as skatole on the basis of gas chromatographic and spectro-photometric characteristics. Preliminary examination of some of the other peaks indicate the presence of one or more aromatic esters, carbazole-like compounds, and an aromatic aldehyde.

REVIEW BY M. L. GILFOYLE

The residue from the nitromethane soluble portion of the neutral fraction of smoke condensate from 5000 domestic cigarettes was investigated. The infrared spectrum of the residue indicated an ester plus an -OH or NH band at 3500cm<sup>-1</sup>.

The residue was gas chromatographed under the following conditions:

Column: 4' x  $\frac{1}{8}$ " - 0.25% SE-30/Glass Beads  
Column Temperature: 65°C - 300°C at 4°C/min.  
Flow Rate: 60 ml/min. He  
Detector Temperature: 315°C  
Injection Port Temperature: 325°C

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The gas chromatogram showed twenty peaks, of which there were two major peaks. The peak eluted at 140°C was skatole and the peak eluted at 185°C was benzyl benzoate, which had not been identified in smoke prior to this time.

The infrared analysis of the eluant at 185°C indicated an aromatic ester. The mass spectrum showed a compound with molecular weight 212. Based on retention time studies, hydrolysis, mass spectrometric and infrared data, this ester was identified as benzyl benzoate.

The following is the elution pattern of nitromethane subfractions off alumina:

↓ Hydrocarbons  
Esters, Ethers  
Skatole and other Methyl Indoles  
Indole  
Carbazoles (methyl carbazoles)  
↓ Resinous Materials

All fractions were gas chromatographed. The latter four fractions were run under the following conditions:

Column: 10' x  $\frac{1}{8}$ " - 20% SE-30/Chromosorb W

Column Temperature: 240°C

Flow Rate: 60 ml/min. He

Detector: Flame ionization

Detector Temperature: 290°C

Injection Port Temperature: 260°C

<u>Retention Time</u>	<u>Components</u>
1 minute	carbazole
2½ minutes	skatole
3 minutes	indole
10 minutes	methyl carbazole
14 minutes	dimethyl carbazole

Composition of Nitromethane Subfraction

<u>Compound</u>	<u>Criteria for Identification</u>
Skatole	IR, UV, GC, Ehrlich Test
Methyl Indole	IR, MS
Dimethyl Indole	IR, MS
Trimethyl Indole	IR, MS
Indole	GC
Carbazole	IR, UV, GC, MS
Methyl Carbazole	IR, UV, GC, MS
Dimethyl Carbazole	IR, UV, GC, MS
Benzyl Benzoate	IR, UV, GC, MS, Hydrolysis
Benzyl Cinnamate	IR, MS
Aromatic Ether (known but not reported)	IR, MS

The author reported 4  $\mu$ g of benzyl benzoate per cigarette and a large amount of skatole, but this was not measured.

SOME KETONES AND PHYTYL ESTERS  
FROM TURKISH TOBACCO SMOKE.  
Alan Rodgman and Lawrence C.  
Cook, Research Department  
R. J. Reynolds Tobacco Company,  
Winston-Salem, North Carolina.

ABSTRACT

Turkish tobacco smoke condensate was fractionated by liquid-liquid partition, crystallization, and column chromatography to yield three fractions designated as Fraction A, B, and C. Vapor-phase chromatography of Fraction A gave as the major component an eighteen-carbon ketone whose VPC emergent time and infrared and mass spectral data were in agreement with those of hexahydrofarnesyl acetone (phytone). Vapor-phase chromatography of Fraction B gave three main carbonyl components. The first to be eluted was a colorless oil whose VPC emergent time and infrared, ultraviolet, and n.m.r. spectral data were in agreement with those of the thirteen-carbon ketone 2-methyl-5-isopropyl-1, 3-nonadien-8-one (solanone). The other two VPC fractions are ketones whose identities to date are unknown. Infrared absorption study indicated that Fraction C was an unsaturated ester. Saponification yielded the diterpenoid alcohol phytol plus an acid fraction. Treatment of this acid fraction with diazomethane yielded a methyl ester fraction. Column chromatography, VPC, and mass spectrometric study of this methyl ester fraction before and after catalytic hydrogenation indicated the presence of the methyl esters of the saturated C<sub>11</sub> to C<sub>24</sub> acids, inclusive; the monounsaturated C<sub>15</sub> and C<sub>18</sub> acids; the diunsaturated C<sub>18</sub> acid; the triunsaturated C<sub>13</sub>, C<sub>14</sub>, C<sub>16</sub>, and C<sub>18</sub> acids; plus the possible presence of the methyl esters of some branch-chained acids. The ratio of saturated acids:unsaturated acids in the methyl esters was about 2:1. Of the saturated acids, myristic (C<sub>14</sub>), palmitic (C<sub>16</sub>), heptadecanoic (C<sub>17</sub>), and stearic (C<sub>18</sub>) acids were the most plentiful; of the unsaturated acids, pentadecenoic (C<sub>15</sub>), an unsaturated C<sub>16</sub>, oleic (C<sub>18</sub>), linoleic (C<sub>18</sub>), and lino-  
lenic (C<sub>18</sub>) acids were the most plentiful.

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THE USE OF 3-METHYLBENZO-  
THIAZOLONE HYDRAZONE HYDRO-  
CHLORIDE FOR THE ESTIMATION  
OF ALIPHATIC ALDEHYDES IN  
CIGARETTE SMOKE.

Dr. A. S. Weaving, The Imperial  
Tobacco Company, Limited,  
Bristol, England.

ABSTRACT

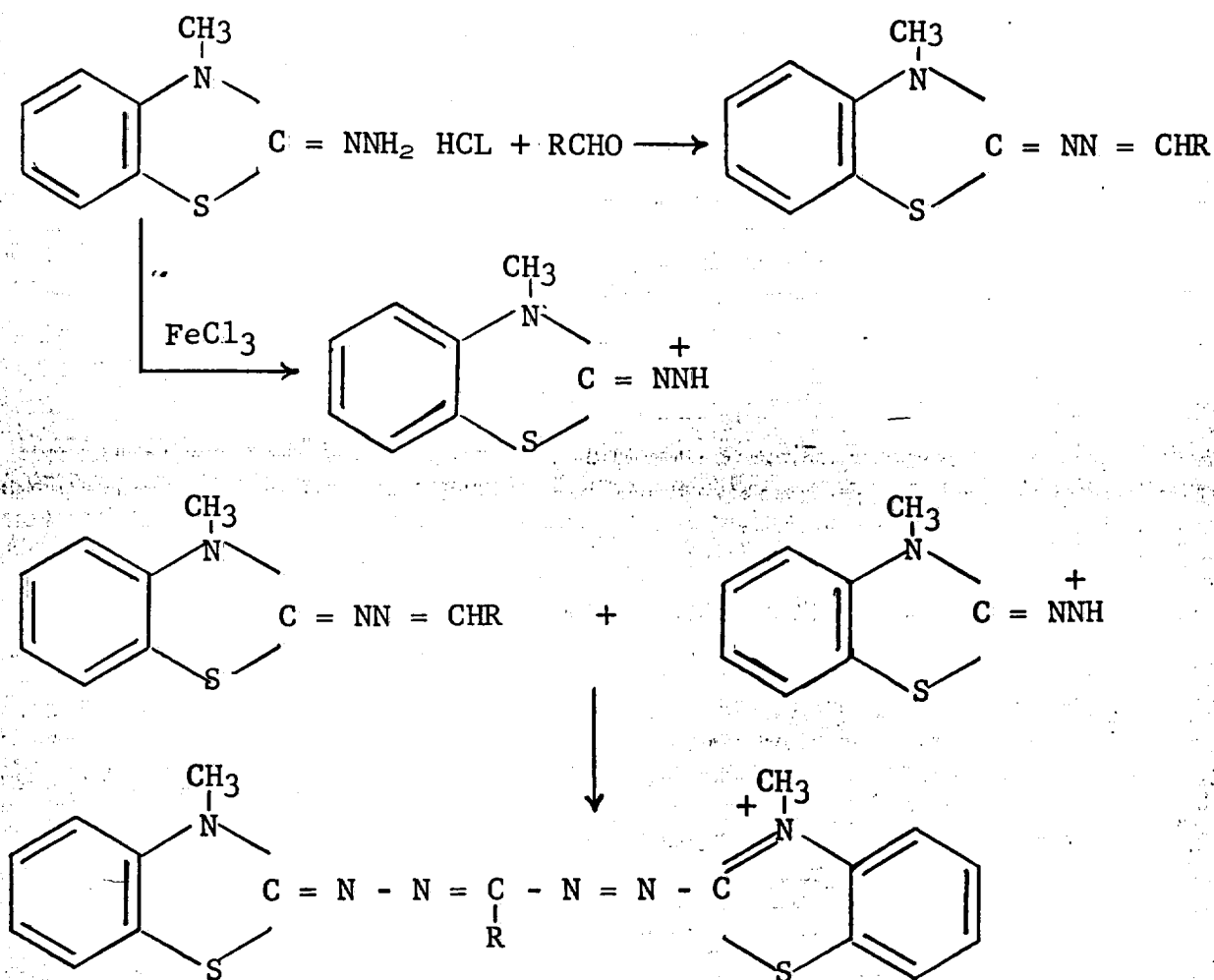
A colorimetric method for the determination of aliphatic aldehydes in cigarette smoke is described. The method is essentially that used by Sawicki *et al* (*Anal. Chem.*, 1961, **33**, 93) for the analysis of these compounds in air and motor exhaust fumes. The reagents used are 3-methylbenzothiazolone hydrazone hydrochloride (3-MBTH) and ferric chloride. Aldehydes are converted into highly coloured tetraazopentamethine cyanine dyes but ketones form azines which do not react with the ferric chloride oxidised form of 3-MBTH to form dyes. Furfuraldehyde does not react and compared with acetaldehyde, benzaldehyde gives a very low colour yield. Reproducibility of the method for several smokings of the same cigarettes is very good and duplicate determinations on the same smoke extract are excellent. Results obtained with and without a Cambridge filter between the cigarette and the trap are discussed. The possible interference with the method of other compounds in cigarette smoke is considered.

REVIEW BY R. A. LUTZ

A rapid and specific colorimetric method for the determination of aliphatic aldehydes was presented. An intense color develops from the reaction of 3-Methylbenzothiazolone Hydrazone Hydrochloride (3-MBTH) and  $\text{FeCl}_3$  with aldehydes. The reaction takes place in three stages as shown:

1. Condensation to azine
2. Reactive cation formation
3. Both react together to form a highly colored dyestuff

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Ketones form azines which do not react with the ferric chloride oxidized form of 3-MBTH to form dyes. Phenols, such as skatole, carbazoles, and other indoles, interfere or are detected by the reagent. Furfuraldehyde does not react. Other aldehydes give color relative to acetaldehyde as follows:

<u>Compound</u>	<u>Color Relative To Acetaldehyde</u>
Acetaldehyde	1.00
Formaldehyde	.94
Propionaldehyde	.96
n-Butyraldehyde	.98
iso-Butyraldehyde	.72
m-Valeraldehyde	.66
iso-Valeraldehyde	.54
n-Hexaldehyde	.48
Acrolein	.43
Crotonaldehyde	.40
Glyoxal	.40
DL-Glyceraldehyde	.84
Pyruvaldehyde	.48
Benzaldehyde	.05

The details of the method are reported in the following references:

M. Uneda J. Pharm. Soc., Japan, 83, 951-956 (1963).

E. Kamata, Bull. Chem. Soc., Japan, 37 (11), 1674-1677 (1964).

DETERMINATION OF CATION AND ANION  
CONCENTRATIONS IN TOBACCO AND  
CIGARETTE SMOKE.Paul F. Taft, United States Tobacco  
Company, Nashville, Tennessee.ABSTRACT

Ion exchange techniques have been applied to tobacco and cigarette smoke to determine cation and anion concentrations. An aliquot from the water extract of the tobacco sample was passed through a cation exchange resin and titrated with standard base. Another aliquot was passed through an anion exchange resin and titrated with standard acid. Smoke was collected from five cigarettes smoked 47 mm each. The cigarettes were smoked on a machine designed to take a 35 cc puff of 2 second duration once each minute. One sample was collected in excess base, passed through a cation exchange resin, freed from  $\text{CO}_2$  and titrated with standard base. Another sample was collected in standard acid, passed through an anion exchange resin and titrated with standard acid. In all cases, the number of equivalents of standard base is equal to the number of equivalents of anion present and the number of equivalents of standard acid is equal to the number of equivalents of cation present. Details of the methods and typical results for tobacco and cigarette smoke will be given.

REVIEW BY H. C. SILBERMAN

The titration curves are very flat, which is characteristic for highly buffered solutions. The titration method was tried out first on synthetic samples containing known amounts of acids, neutrals, and bases. Phenols were not used in synthetic mixtures because they are present only in very small amounts in tobacco smoke.

The tobacco samples were blended with water in a Waring Blender. The tobacco cations and anions were titrated separately. A result is shown below:

<u>Tobacco Sample</u>	<u>m. eq. Cation/gm</u>	<u>m. eq. Anion/gm</u>
No. 1	2.990	2.539
No. 2	2.914	2.427
Average	2.952	2.483

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Various results for tobacco and smoke analyses were given, but the samples were never defined other than by a code.

The author felt that his method is simple and gives good data in a reasonable amount of time.

Paper No. 33

GAS CHROMATOGRAPHIC DETERMINATION  
OF THE WATER IN CIGARETTE MAIN-  
STREAM SMOKE AND TOTAL PARTICULATE  
MATTER.

Frederick A. Thome, R. J. Reynolds  
Tobacco Company, Winston-Salem,  
North Carolina.

ABSTRACT

A method has been developed for determining the total water content of cigarette smoke and of cigarette filter media. Pyridine was employed as a water-absorbing solvent, the water content of the pyridine being measured by gas chromatography before and after contact with the smoke or filter media. The water has been determined in the mainstream smoke of burley, flue-cured, Turkish and blended tobacco rods. The mainstream water content has also been determined for blended tobacco rods with and without cellulose acetate filters. For the above samples, the water content of the total particulate matter (T.P.M.) of the smoke was also determined using the same gas chromatographic technique. The T.P.M. was considered that part of the smoke which was collected on a Cambridge filter mat positioned directly behind the cigarette. Data are presented showing the total water content of the mainstream smoke of filtered cigarettes after they had been conditioned to tobacco moisture levels ranging from 0.7% to 16.6%.

REVIEW BY W. J. NEDLOCK

The procedure followed for determining the water content of mainstream smoke is to smoke five cigarettes into a trap, using a Bradford falling water smoking machine. The smoke is bubbled through pyridine contained in a trap and through two scrub traps. The equipment is dismantled and washed with the pyridine from one scrub trap. The pyridine is then transferred to a 50 ml. volumetric flask, brought to volume, and capped with a septum.

The author emphasized that the reasons for using pyridine were its hygroscopic properties, its non-water producing effect in reaction with smoke, its infinite resolution, and its enhanced peak height in relation to the concentration of pyridine applied to the Chromatograph.

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Water content is determined chromatographically using a constant volume injection technique. The chromatograph is a Perkin-Elmer 150, and the column is a 1/4" by 4' copper tube packed with 50/80 byalate H. The water content is determined from peak height, which gives a linear relationship with weight of water present in the pyridine.

The water content in TPM is determined by smoking five cigarettes on a Cambridge filter pad and extracting the pad for 15 to 20 minutes in 10 ml. of pyridine.

The total water content of cigarette mainstream smoke for a number of blends is given in Table 1, and the water in the TPM of cigarettes of blended tobaccos is given in Table 2. Table 3 shows water in mainstream smoke and TPM as a function of moisture content of tobacco.

TABLE 1

TOTAL WATER IN CIGARETTE MAINSTREAM SMOKE

<u>Cigarette Sample</u>	<u>Description of Rod and Filter</u>	<u>Total H<sub>2</sub>O mg/5 cig</u>	<u>H<sub>2</sub>O mg/cig</u>
A - Blended Tobacco	70 mm rod	32.0-41.0*	7.4-8.2*
	15 mm acetate filter	39.5**	7.9**
B - Blended Tobacco	70 mm tobacco rod	54.5-63.5	10.9-12.7
		59.5	11.9
C - Blended Tobacco	85 mm tobacco rod	45.4-48.0	9.2-9.6
		47.5	9.5
D - Blended Tobacco	80 mm tobacco rod	42.5-43.5	8.5-8.7
		43	8.6
E - Blended Tobacco	10 mm acetate filter		
	10 mm bonded carbon filter	33.5-36.0	6.7-7.2
	65 mm tobacco rod	34.0	6.8
Flue-cured	70 mm tobacco rod	68-86	15.6-17.2
		81.5	16.3
Burley	70 mm tobacco rod	46.3-49.0	9.3-9.8
		47.5	9.5
Turkish	70 mm tobacco rod	73-73.5	14.6-14.7
		73.3	14.6
Cellulose	70 mm rods	68-81	13.6-16.0
		74.7	14.9

\* range    \*\* average

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TABLE II

## WATER IN TPM OF CIGARETTES OF BLENDED TOBACCO AND TOBACCO TYPES

Sample Description	Butt Length After Smoking	mg H <sub>2</sub> O in TPM/cig	mg TPM per cig	% H <sub>2</sub> O in TPM/cig	% of Total H <sub>2</sub> O in TPM
A - 85 mm Blended Tobacco Rod	35 mm	2.1	26.6	7.9	25.3
B - 70 mm Blended Tobacco Rod	20 mm	2.7	30.4	8.8	22.7
C - 80 mm Blended Tobacco Rod	30 mm	2.0	23.4	8.5	23.3
D - 70 mm Tobacco Rod 15 mm Acetate filter	35 mm	1.8	18.2	9.9	23
Burley Rod - 70 mm	20 mm	2.7	24.9	11	28.5
Flue-cured Rod - 70 mm	20 mm	3.0	33.1	9.1	
Turkish Rod - 70 mm	20 mm	2.4	27.5	9.0	16.4

TABLE III

## WATER IN CIGARETTE\* MAINSTREAM SMOKE AND TPM AS A FUNCTION OF MOISTURE CONTENT OF TOBACCO

Percent Moisture in Tobacco	Total Mainstream Smoke Water, mg/cig	Water in Cigarette TPM, mg/cig	Percent of Total Water in TPM	Water in TPM as Percent of TPM
0.7	3.3	0.5	15.0	2.9
5.5	4.9	0.8	16.3	4.2
8.0	5.6	0.9	16.0	5.7
9.1	6.1	1.1	18.0	6.6
13.4	7.8	1.7	21.8	8.9
16.4	9.6	2.2	23.0	10.9

\* 70 mm Rod with 15 mm acetate filter

Paper No. 34

THE CAPILLARY PRESS, A SMOKING  
MACHINE FOR PREPARING INSTANT  
SMOKE CONDENSATE.

F. Seehofer and D. Hanssen,  
B.A.T. Cigaretten-Fabriken  
G.M.B.H., Hamburg/Forschung  
und Entwicklung.

ABSTRACT

This paper describes the construction and working principle of an automatic capillary press, a smoking machine for preparing instant smoke condensate from 15 cigarettes per smoking procedure. The smoking capacity is 500 cigt/day. Condensate production by means of total yield, as well as by means of individual puff yield, is possible. The reproducibility of smoke condensate and smoke nicotine yield is 2.5% RSD.

The smoking machine-vacuum pump-precipitation part allows variation of smoking parameter and precipitation conditions. Consequently, the precipitation system can be substituted as desired. In all cases free or restricted smoking is possible.

Changes in the results of total yield and individual puff yields as a function of initial tobacco moisture content as well as different precipitation procedures are indicated. The data for free and restricted smoking obtained from the capillary press are compared with those obtained from B.A.T. - 15 channel smoking machine with electrostatic precipitation.

A copy of this paper is available in the library.

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THIN-LAYER CHROMATOGRAPHIC  
SEPARATION OF BENZO(a)PYRENE  
FROM CIGARETTE TAR.

Bernard W. Oliver, Jr.,  
Tennessee Eastman Company,  
Kingsport, Tennessee.

ABSTRACT

The current method of analyzing for benzo(a)pyrene in cigarette tar is time consuming and requires a large sample. Therefore, research was carried out to develop a new method requiring less time and a smaller sample. Two-gram samples of tar were partitioned between cyclohexane and methanol: water (4:1). The cyclohexane phase was extracted with nitromethane, and the benzo(a)pyrene was isolated from the nitromethane extract by thin-layer chromatography. The total amount of benzo(a)pyrene was determined by baseline ultraviolet adsorption, and losses were determined by isotope dilution, using  $C^{14}$ -tagged benzo(a)pyrene. Again by using isotope dilution, benzo(a)pyrene was separated from 200 milligram samples of tar (the amount from about five cigarettes) directly by thin-layer chromatography. The total amount of benzo(a)pyrene was determined by fluorescent spectroscopy, and the  $C^{14}$  benzo(a)pyrene was determined by liquid scintillation spectroscopy. Finally, by using the above technique, benzo(a)pyrene was separated from the tar from single cigarettes. It was found that thin-layer chromatographic separations of tobacco tar are adequate to produce benzo(a)pyrene of sufficient purity that it can be quantitatively analyzed by spectroscopic means. It was also found that the analysis for benzo(a)pyrene in the tar from one cigarette is as precise as the analysis of benzo(a)pyrene in two-gram samples of tar. The use of thin-layer chromatography and the sensitivity of liquid scintillation spectroscopy and fluorescent spectroscopy therefore permit the relatively precise and rapid analysis of the tar from a single cigarette for benzo(a)pyrene.

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REVIEW BY F. E. RESNIK

The author emphasized that his analysis for B(a)P could be carried out with smoke from one cigarette. This was done by repeated separations using thin layer chromatography followed by fluorescence analysis. The  $C^{14}$ -labelled B(a)P was used to correct the recoveries in the separation steps. The recovery values ranged from 17.3% to 51.2%. One important point emphasized by the author was that the B(a)P content of handmade cigarettes increased more than twice over the B(a)P content of control machine-made cigarettes. The B(a)P values for handmade cigarettes were 2.69 and 3.03 parts/million vs. 1.23 and 1.47 for the machine-made control. The standard deviation on these analyses was  $\pm 27\%$  absolute at the 1.7 parts/million level at 95% confidence.

A copy of this paper is available in the library under the title, "Determination of Benzo(a)pyrene in Cigarette Tar by Thin-Layer Chromatography and Fluorescence Spectroscopy."

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*Carpenter*

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